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CATTLE PLAGUE ANTI-SERUM PREPARED BY INTRARUMENAL HYPERIMMUNISATION.

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THE hyperimmunisation of cattle for producing cattle plague antiserum is by any of the established techniques a brutal procedure, and even to the most callous operator reduced efficiency on account of surgical complications is a constant source of anxiety. Although " immune " serum may be quite useful, and is, in fact, sometimes administered, laboratories engaged in regular large scale production of cattle plague antiserum, with the exception of those in which buffaloes can be employed as serum producers, still find themselves compelled to prepare the bulk of their serum by hyperimmunisation.

The introduction by Hornby (1923) of intrarumenal hyperimmunisation constituted an attempt to reduce objections both on æsthetic and on surgical grounds, but it is noteworthy that although cattle plague antiserum so prepared has since then been exclusively and successfully used in Tanganyika Territory the process has engaged little attention in other countries.

When instituting the preparation of cattle plague antiserum in the Sudan a few years ago, it was decided to study the intrarumenal process as early as possible, but it was considered first desirable to establish a method that had received more general approval, and, having found hyperimmunisation unavoidable, the intramuscular route was adopted as being the least objectionable while producing serum of no less potency than other methods.

Subject to certain local limiting factors, intramuscular hyperimmunisation has during a period of seven years proved satisfactory in the Sudan, and in any case the objections that may be made are not on account of inadequate potency of the resultant serum. A

single serious attempt only has been made to prepare serum by intrarumenal hyperimmunisation, and since further attempts are not contemplated it seems desirable to record the results although they are not very comprehensive.

Before discussing the intrarumenal process, it is necessary briefly to outline the local intramuscular technique. The principle is hyperimmunisation on the most intensive scale that local cattle will tolerate. This principle is fairly general, although actual details of technique are subject to variation in different countries. A very common procedure is to give a maximal dose of virulent cattle plague blood, to practise a number of bleedings—usually from four to six—at approximately weekly intervals, and then to allow the cattle to rest for an appreciable time before re-hyperimmunising and repeating the cycle.

In the Sudan this type of procedure would be unsuitable. For the sake of grazing, serum is prepared in the Southern Sudan, but climatic conditions, particularly heavy rain and extensive flooding, make it impossible to work for more than half the year. The whole serum-making process has, therefore, to be intensified in order to practise as many hyperimmunisations and bleedings as possible within the available time. At present, serum producing cattle are hyperimmunised every 25 days, with two bleedings in between, and the maximum tolerated injection rate of virus (citrated cattle plague blood) has for this interval been determined at about 2 c.c. per 1 lb. body weight, but not all the animals tolerate this dosage throughout the season. Six or seven hyperimmunisations thus constitute a season's maximum, and it has been found that the resultant serum is of adequate potency, while special tests have shown that it does not appreciably vary in titre from bleeding to bleeding.

The foregoing brief description of technique is not set out for discussion, but is necessary for adequate appreciation of the results obtained with serum obtained by intrarumenal hyperimmunisation. The latter need be compared with the Sudan " routine " serum only in relative terms, but it is desirable also to set out details that will allow it to be compared in more absolute terms with serum prepared in Tanganyika by the same process.

Finally, it must be pointed out that the qualities of " immune " serum cannot confuse the results of tests of Sudan " hyperimmune " serum. Owing to the necessity for commencing any season's serum production with no delay on account of preliminary operations, the cattle destined to be serum producers for the ensuing season are collected and immunised towards the end of the current season. There is thus an interval of about six months before their first hyperimmunisation, which will permit of the disappearance of any protective value that their serum may have acquired as the result of preliminary immunisation.

The details of the single test of serum prepared by intrarumenal hyperimmunisation are as follows :—

Twelve cattle of 700 to 750 lb. weight which had been immunised

about six months earlier were selected at random from a group of cattle destined for routine serum production. They were injected on the same days and with the same virus as the others, and were bled at the same rates on the same days. The only difference throughout the season was that, instead of their hyperimmunising injections consisting of citrated virulent blood at the rate of 2 c.c. per 1 lb. body weight intramuscularly, the virus was given intrarumenally at the rate of 4 c.c. per 1 lb. body weight, *i.e.*, double the " routine " dosage (actually, for the sake of convenience, a flat-rate dosage of 3 litres was given). Double dosage for intrarumenal administration was adopted in pursuance of one's established principle of maximal hyperimmunisation, since the cattle had been found capable of tolerating this dosage without developing more than transient symptoms of malaise.

Five cycles of hyperimmunisation and bleeding were completed, and the pooled serum obtained after each intrarumenal injection was separately stored. The parallel serum prepared by intramuscular hyperimmunisation was pooled to make a single sample, since it was already known from earlier tests that the titre did not appreciably vary from bleeding to bleeding.

A potency test was carried out with the two classes of serum, but, whereas the single sample prepared by the routine method was tested in a range of doses, the five samples prepared by the intrarumenal process were tested only at the " average " dosage of 10 c.c. per 100 lb. body weight. It was intended that if, at this " average " dosage, the experimental samples proved satisfactory a second titration in a range of doses should be performed. As will be seen, they were of such low value that no further test was indicated. The details of the actual test are shown in Table I.

As a preliminary to the very little discussion demanded by the results set out in the Table, it is relevant to mention that the mixed sample of serum obtained by intramuscular hyperimmunisation was not the only one tested during the season. This particular sample is included in the Table because it was tested *simultaneously* with the experimental samples, and the cattle employed in the test were selected at random from one batch of arrivals. In the course of the season three other tests of routine serum were carried out :—

(*a*) A sample consisting of serum the bulk of which was obtained after the first, and a little after the second, intramuscular hyperimmunisation. The result, apart from the brand numbers on the cattle, might have been that shown in the Table.

(*b*) A sample obtained mainly from third hyperimmunisation, with a little from the second and the fourth. This was tested on five cattle at the 10 c.c./100 lb. dosage only ; two cattle developed mild thermal reactions and three no symptoms.

(*c*) A sample containing serum from fourth, fifth and sixth hyperimmunisations. Tested exactly as (*b*), three cattle showed mild thermal reactions and two no symptoms.

TABLE I.

11.5.32. Test of Samples of Cattle Plague Antiserum prepared by Intrarumenal and Intramuscular Hyperimmunisation.

Process	*Hyper-immunisation.*	*Dose per 100 lb.*	*Bull.*	*Symptoms.*	*Result.*
Intrarumenal ...	1st	10 c.c.	28	No symptoms ...	... Survived
			29	Severe cattle plague	... Survived
			30	Severe cattle plague	... Died
	2nd	10 c.c.	31	Mild cattle plague	... Survived
			32	No symptoms ...	... Survived
			33	Severe cattle plague	... Survived
	3rd	10 c.c.	34	No symptoms ...	... Survived
			35	Severe cattle plague	... Died
			36	No symptoms ...	... Survived
	4th	10 c.c.	37	Severe cattle plague	... Died
			38	Severe cattle plague	... Survived
			39	Severe cattle plague	... Died
	5th	10 c.c.	40	Severe cattle plague	... Survived
			41	No symptoms ...	... Survived
			42	Severe cattle plague	... Died
Intramuscular ...	Mixed	5 c.c.	43	Severe cattle plague	... Died
			44	Mild cattle plague	... Survived
			45	Thermal reaction only...	Survived
	„	10 c.c.	46	Mild thermal reaction only	... Survived
			47	No symptoms ...	... Survived
			48	Mild thermal reaction only	... Survived
	„	15 c.c.	49	No symptoms ...	... Survived
			50	No symptoms ...	... Survived
			51	Mild thermal reaction only	... Survived
Controls		Nil	330	Severe cattle plague	... Died
			331	Severe cattle plague	... Died
			332	Severe cattle plague	... Died

It is thus evident, as had been established in earlier seasons, that the routine serum, whether from early, middle, or late bleedings, would at the 10 c.c./100 lb. dosage prevent the development of any symptoms other than mild fever. The single test of the samples prepared by intrarumenal hyperimmunisation shows that none of them were up to this standard, and, moreover, that the serum obtained after several hyperimmunisations was no better than that obtained after the first.

A more complete condemnation of the intrarumenal serum lies in the fact that some of the test cattle were undoubtedly insusceptible to cattle plague ; it is impossible to be sure of the actual number, but judging from the records of virus producers throughout the season,

which in this respect was a bad one, it would not be excessive to estimate them at 20 per cent. With this knowledge it becomes evident that for a true assessment of the relative serum potencies the cattle which must particularly be studied are those which developed some kind of reaction when given serum and virus. In the case of serum prepared by intramuscular hyperimmunisation no beast receiving 10 c.c. per 100 lb. body weight developed more than mild fever, whereas all beasts that developed symptoms when tested with the same dose of serum prepared by the intrarumenal process developed advanced symptoms of cattle plague and several of them died.

Cattle plague antiserum prepared by intrarumenal hyperimmunisation has thus shown itself to be markedly inferior to that prepared by the more generally practised intramuscular process. The inferiority is the more noteworthy in that, with complete parallelism in regard to all other items of technique, double the quantity of virus was administered at each hyperimmunising injection. Furthermore, in order to justify some kind of absolute comparison with the technique employed in Tanganyika, it must be especially noted that our flat-rate dosage of 3 litres of virus per hyperimmunisation was more than the Tanganyika rate of 2 litres ; in addition, our hyperimmunising injections were repeated at short intervals (25 days), whereas by the Tanganyika technique the intervals are much longer (never less than five weeks and sometimes more). It is conceivable that if the preliminary potency test had shown a less marked inferiority, or if the hyperimmunising injections had been practised by administering less virus than was actually given, further efforts to adapt the intrarumenal technique might have been contemplated. The very marked inferiority in spite of more intensive hyperimmunisation has, however, led to the decision that, in the Sudan at any rate, the intrarumenal process shall receive no further attention.

It is necessary, before finally abandoning discussion of the method, to consider why cattle plague antiserum prepared by the intrarumenal technique has proved satisfactory for so many years in Tanganyika Territory. When first briefly reporting (*Annual Report, Sudan Veterinary Service*, 1932) the low potency of such serum in the Sudan, it was suggested that an explanation was afforded by the strong resistance of susceptible Tanganyika cattle to the course of the disease. Study of the Animal Reports of the Veterinary Pathologist of Tanganyika Territory for the last ten years shows that the cattle used for testing the efficacy of serum are susceptible to cattle plague in sufficiently high proportion but that, as compared with susceptible cattle elsewhere, an extraordinarily large proportion of " controls " recover after developing cattle plague in varying degrees of severity. This state of affairs is far more detrimental to serum testing than possession of a number of completely immune cattle in a population in which a high mortality rate occurs in those stil' susceptible. This fact has been realised in Tanganyika Territory and it has been decided that the usual type of serum potency titration employed in other

countries is unlikely to afford sufficiently clear-out results to be of much use in Tanganyika. In consequence, a special method of serum testing has been devised in which a consecutive series of small tests is carried out with a standard dosage of serum and a final assessment is made in consideration of the aggregate of results. The standard for " good " serum naturally implies that no serumised beast shall die of cattle plague when simultaneously injected with virus, but apart from this factor the potency can only be tentatively assessed by comparing the severity of symptoms developed in the total number of serumised and unserumised animals. Without discussion of the details of the actual technique employed in carrying out this type of potency test, it is certain, from the very fact that such a method of testing has been adopted, that a serum of low potency will possess adequate protective power for Tanganyika requirements.

Mention has already been made of the lack of attention given to intrarumenal hyperimmunisation in other countries. In the available literature only one further reference to it, by Hall (1929), can be found. This worker, who has had the advantage of experience in Tanganyika Territory, attempted to prepare cattle plague antiserum by this process in Nigeria. As in our own case, he did not proceed beyond the preliminary stages. No protocols are provided, but it is concluded that in Nigeria " we do not feel justified in either introducing the intrarumenal method of production of serum or even allowing a proportion of the serum obtained by this method to be pooled with the serum obtained by intramuscular hyperimmunisation." In a later publication, Hall (1933) again mentions the method and advances the opinion that " the good results obtained in Tanganyika Territory can only be attributed to the natural resistance the cattle of that country possess against rinderpest."

Summary.

An attempt has been made in the Sudan to prepare cattle plague antiserum by intrarumenal hyperimmunisation of cattle. The dosage of virus was double that employed in parallel hyperimmunisation of other cattle by the intramuscular route, and was not only 50 per cent. larger than the dosage employed in Tanganyika Territory but was repeated at shorter intervals. The resultant serum was markedly inferior to that prepared by the intramuscular process, being, in fact, quite incapable at the usual dosage of protecting Sudan cattle against a simultaneous injection of virus. Furthermore, the serum obtained after four or five hyperimmunisations was of no higher potency than that obtained after the first one.

The satisfaction that serum obtained by the intrarumenal process gives in Tanganyika Territory appears to be referable to an unusual morbidity—mortality ratio in the face of cattle plague infection in that country. Although the morbidity rate is high, the natural resistance of the cattle is also so high that the mortality rate is very

low, and an antiserum of low potency has sufficient protective qualities to give local satisfaction.

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CATTLE DISEASES OCCURRING IN PALESTINE AND SEGREGATION AND PREVENTION CONTROL OF BOVINE CONTAGIOUS ABORTION

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PALESTINE is a small country, having approximately the same area as that of Wales. The importance of a country, however, is not entirely dependent on its size when considered historically, politically, or, especially, from a point of view of intensive cultivation or production. In form, the country is roughly oblong, with a coast-line of about 150 miles. To the east it is bounded by the River Jordan and the Dead Sea and a strip of desert which divides it from Trans-Jordan. To the south it fades off into the Sinai desert, across which the railway continues from El Arish to Kantara on the Suez Canal, some 15 miles south of Port Said. Syria forms the northern border. The country in this neighbourhood is hilly, rising to 3,960 feet at Jabel Jarmuk, the highest point in Palestine. These hills extend southwards nearly to Beersheba, leaving a coastal and southern plain of varying width. The most fertile land consists of this coastal plain and its extensions. To the north the plain of Esdraelon bisects the hill country from Haifa to the Jordan valley just south of Lake Tiberias.

Animals imported by sea must pass through the port of Haifa, where a large harbour has recently been completed, and enter a well built quarantine. Fourteen small quarantine stations have also been placed at different points around the land borders. The total annual revenue from this source is £7,600.

The approximate animal population of Palestine is as follows:—

Native cattle	160,000
Dairy cattle	14,000
Sheep	250,000
Goats	400,000
Horses	14,000

The staff of the Veterinary Service, which includes Animal Husbandry and is part of the Department of Agriculture and Forests, consists of a Chief Veterinary Officer, a Senior Veterinary Officer and an Assistant Senior Veterinary Officer. The remaining qualified staff which are locally recruited includes six Veterinary Officers and six Assistant Veterinary Officers.

There are also 44 qualified Veterinary Surgeons in various forms of practice or employment, including Municipalities. Their qualifications have been obtained in a great many different countries. Unqualified Government-staff are employed at Quarantine Stations and as Stock Inspectors and Inoculators.

The population of the country according to the Census of 1931 is approximately as follows:—

(1) Arabs, Moslems	760,000
(2) Arabs, Christians	90,000
(3) Jews	175,000

A comprehensive Veterinary Surgeons ordinance was approved and published in 1930 and all qualified Veterinary Surgeons must be licensed.

The Arab population, apart from the towns, chiefly live in villages. A wandering population, largely consisting of Bedouins, amount to some 60,000. These people mostly live in tents and move with their flock and camels in search of grazing.

There are now a large number of Jewish settlements, all of which are engaged in various forms of agriculture. Many of these are wholly engaged, or partially so, in dairying.

The settlements themselves are in many cases communal, and in these the land and cattle are common property. Others consist of individualist groups in which cattle are separately housed by each owner.

There are also four or five German settlements which were colonised by Germans many years ago. These settlers are frugal and hard working and have paid their way. Small colonies of Germans also exist in the three main towns of Jerusalem, Jaffa and Haifa.

The present retail price of milk is about 2s. 6d. per gallon, while the producer receives about 1s. per gallon. These prices are considerably lower than those charged a few years ago.

Types of Cattle.—The common or native cattle of Palestine are of poor type. Subjected to periodical starvation during the long dry summer, and infected with various types of tick fever to which they have become very resistant, they remain stunted and weedy. The average weight of the adult cattle is about 700 to 800 lbs.; the milk yield is negligible and their chief use is for the production of ploughing oxen. There is no attempt to improve breeding, the young bulls remaining with the herd for service until of sufficient growth to be used for ploughing.

At the commencement of the present administration dairy cattle consisted of Damascus or Beyrouth animals with at times a mixture of Holland blood provided by a few imported bulls. A few other European types had also been imported. With the increase in Jewish immigration it became necessary for the numbers of dairy cattle to be increased. At first Holland cattle were largely imported, but a disregard of precautions against tick infections led to heavy mortality. By keeping the cattle from pasture and the use of dip even as a wash, this mortality is reduced to small proportions, and herds of Holland cattle are still maintained at some settlements. The Damascus or Beyrouth cattle are also popular and are somewhat more resistant to tick fevers though not to the same extent as are native cattle. They give a good milk yield.

It is difficult, however, to purchase sufficient numbers as the export from Syria is prohibited except by special permit. Apart from small numbers of other breeds, the remaining cattle consist of crosses of these types, the Holland Damascus cross being quite popular. About 100 milking cattle from Switzerland have recently been imported.

The value of these breeds in Palestine are as follows:—

Holland	£40-£60
Damascus or Beyrouth	£25-£40
Crosses (pure-bred)	£30-£45
Native	£5-£10

An attempt to produce resistant dairy stock has also been made by crossing Holland bulls with native cows. The first cross gives a good class of cow, small and cheap to feed and with good milk yield.

Subsequent crossing, however, does not maintain the standard—an outcome that has been experienced elsewhere. It may be stated that the settlers do not take kindly to advice and prefer to work out their own problems both in connection with breeding and disease. Rigid disease control, however, is provided under a comprehensive Diseases of Animals Ordinance.

The figures of the Hackalaith, a Jewish Co-operative Insurance Society, for 1932, are of interest with regard to the wastage due to deaths or slaughter of dairy cattle. These show that insurance has been paid on 4½ per cent. of the cattle, while deaths due to tick fevers comprise a little more than 1 per cent. Tuberculosis is very uncommon and only one case of Johne's disease has so far been discovered.

I.—Common Diseases of Cattle.

Epizootic Diseases.

Rinderpest.—Just prior to the commencement of the present Administration in 1920 some cattle plague had been in evidence. From this time until 1926 no cattle plague had occurred. In this

year a new epidemic appeared, spreading rapidly from Turkey and Irak into Syria. For some time it did not pierce the quarantine, but eventually it appeared in a few villages and by the movement of cattle was spread rapidly to localities widely separated. Very energetic measures were introduced, including the stoppage of movement of cattle, and of other animals by special permit. To prevent evasion of the order it was found essential also to prohibit the slaughter of all local stock and to impose severe fines or imprisonment on offenders.

The disease itself was attacked by the " serum alone " method, with slaughter of sick animals and those in-contacts which showed an increased temperature.

In this manner the disease was eradicated in two months. Thirty-nine villages had been infected and 359 cattle died or were destroyed. The total cost of the campaign, which covered practically the entire country except Gaza and Beersheba, including purchase of serum and additional equipment, compensation for animals destroyed and payment of additional technical and clerical staff, amounted to about £12,000.

With the exception of a small recurrence in four villages a few months later and which was disposed of with equal promptness, no further outbreak has occurred, and any future epidemic would tend to follow some fresh wave of virulent infection from Turkey, Iraq, or Persia.

(2) *Foot-and-Mouth Disease.*—Foot-and-Mouth Disease broke out in June, 1931. It had not previously been seen since 1919, except for an isolated outbreak in 1926. It spread rapidly despite energetic measures of control and quarantine. In infected localities artificial transmission of the disease was practised and over 27,000 cattle were so treated. The disease continued to spread, however, throughout the summer and autumn months, but gradually subsided during the winter when all restrictions were removed.

In 1932 the disease broke out again in different parts of the country. It was decided, however, not to introduce general measures of control for the reasons that :—

(*a*) The type of the disease was mild, causing only slight lesions on the mouth and feet, a temporary fever and no mortality.

(*b*) Attempts to limit the spread of this highly infectious disease by all known methods had been of little avail.

(*c*) Past attempts to prevent the introduction of the disease from the neighbouring countries had failed.

(*d*) The imposition of general quarantine restrictions caused great disturbance to the normal life of the peasantry and economic losses which far exceeded those sustained from the disease itself.

The last outbreak occurred on July 24th, 1933, so that the disease has once more subsided.

(3) *Pleuro-pneumonia.*—This disease does not occur in Palestine. A few cases were, however, discovered in Sudan cattle previously imported for slaughter in quarantine. Since that date all import of cattle from infected countries has been prohibited.

(4) *Ephemeral Fever.*—This disease has appeared amongst dairy cattle during one or two summers, but it is confined to a few localities and it not of regular occurrence.

Enzootic diseases.

(5) *Anthrax.*—Anthrax is prevalent throughout Palestine. Severe outbreaks, however, are unusual, the disease being of a sporadic nature and largely conveyed by infected "tibben" (straw). It is still difficult to obtain reports from villagers, though increasing confidence is shown. In 1932 72 outbreaks were reported and 26,000 animals were vaccinated.

(6) *Tick Fevers.*—The actual tick fevers occurring in cattle are as follows and are shown in order of severity :—

Theileria annulata.
Anaplasma marginale,
Babesia bovis.
Babesia bigeminum.

Spirochaetosis and *Trypanosoma theileri* also occur, but are unimportant.

Of these fevers theileriasis causes the greatest mortality. This is very severe if improved cattle are pastured in contact with native cattle and where ticks are prevalent. As already stated, where the cattle are limited in movement and dip is used, the mortality from this cause has been reduced to 1 per cent., though in some years it may be higher. Attacks vary in severity from mild to very acute, and in the latter case are practically always fatal. Native cattle also succumb to this disease or anaplasmosis, but deaths are rare and are frequently associated with periods of starvation.

Anaplasmosis.—This type of fever is also a serious one, and the attack is usually of longer duration. It is caused by *A. marginale,* the *A. centrale* not being known to occur in Palestine. Mortality is not as frequent as in the case of theileriasis.

Babesia bovis.—This infection, while not very common, may be quite a fatal one. It appears to be more prevalent in certain localities. Redwater occurs, but is not a constant symptom, and the parasites are frequently very rare on blood examination.

Babesia bigeminum.—This is the common type of redwater. It is scarcely ever fatal and yields rapidly to treatment with trypan blue. It appears to be economically unimportant.

Mastitis.—Mastitis occurs but is not commonly reported. A streptococcic type is the most common.

Hæmorrhagic Septicæmia.—Outbreaks of this disease, strange

to say, are extremely rare, although they have occurred at long intervals. To meet such an emergency a stock of anti-serum is prepared and maintained at the laboratory.

(7) *Blackquarter.*—This disease occurs throughout Palestine, but its incidence is not high and there is little mortality in outbreaks provided the cattle are immediately vaccinated.

Prophylactic vaccination of young stock is practised in localities where outbreaks have previously occurred.

(8) *Tuberculosis.*—Tuberculosis is comparatively uncommon. During the years 1931 and 1932 four cows were reported to have died and 233 cases were observed in slaughter houses. During the two previous years an investigation had been made of the incidence and distribution of this disease in the course of which 6,784 dairy cattle were subjected to the tuberculin test. Of this number 114 cows or 1·68 per cent. gave a positive reaction and six clinically infected animals only were detected. It would also appear to be very uncommon among native cattle, judging by the negligible number of diseased carcases or organs detected by Municipal Veterinary Surgeons at slaughter houses.

(9) *Johne's Disease.*—In spite of large numbers of dairy cattle having been imported, only one case of Johne's disease has been discovered.

(10) *Coccidiosis.*—Two cases in calves of severe infection with coccidiosis associated with blood stained diarrhœa have recently been diagnosed.

II. Contagious Abortion Control.

During the first two years of the British Administration under the Mandate some investigation had been made into the possible existence of contagious abortion in Palestine. From a clinical point of view no reports were forthcoming as to the existence of abortion as a serious factor in cattle raising. One exception however was brought to notice by the occurrence of a virulent outbreak in a small herd of Syrian milking cattle in Jerusalem. In this outbreak, which was found by laboratory tests to be due to contagious abortion, no contact with imported European cattle could be traced with the exception of a Holland Bull which had been brought from Egypt. The herd was subsequently disposed of by slaughter. It did, however, serve as a warning of the danger of the spread of contagious abortion.

At about this time an urgent demand was arising for improved dairy cattle, and large numbers of European cows, particularly from Holland, were imported. These cows were mostly tested prior to export and a quarantine test was also introduced and positive cows were destroyed. Where, however, numbers of cattle

are imported a test of this nature is insufficient to ward off infection entirely, as agglutinins do not appear in the blood at the time of infection and within the quarantine period allotted some infected animals may be released as negative. Moreover, adequate quarantine facilities were not at the time available.

During the course of the next year outbreaks of abortion began to occur, especially in newly formed settlements. By the end of 1924 some of these outbreaks became so serious that measures of control had to be introduced and better facilities provided for laboratory testing.

Some herds were by this time becoming badly infected, while in others the disease was just commencing and the sale of cattle was further spreading the disease to fresh centres.

Considerable opposition by the settlers to any form of control and apathy in the face of laboratory tests were now experienced. Measures of force are undesirable, as little can be done without complete understanding and co-operation by the settlers. The self-limiting nature of the disease led in some instances to a feeling of false security and to the production of arguments against the necessity for control measures.

In the face of this opposition the testing of herds had to be abandoned, since no effort was being made to isolate or dispose of reactors. One of the first herds in which testing was commenced had at this time only 18 positive cases out of 262 cattle. This settlement is now almost totally infected and the cattle in it have little sale value though previously it was the main centre for breeding for sale. They are still unable to provide the necessary facilities for control measures and so cannot be accepted.

At a later date the losses in some cases were becoming so serious that co-operation and advice was earnestly sought. In some apparently recent outbreaks blood tests revealed a widespread infection throughout the herds and many abortions rapidly followed.

Some preliminary work had already been carried out with a severely infected herd belonging to an Agricultural School in which a new herd was being formed from the segregation of tested calves. Accurate records were maintained as to the respective values of the herds and the progress of the disease and its mode of spread, and the test itself was checked and studied.

This School has recently disposed of the remaining infected animals, and after thorough disinfection of the infected stable, which was vacated for a period of five months, the negative herd was moved into these premises in October, 1932. They have since remained free of the disease.

The following records of the two herds for the period of twelve months ending September 30th, 1929, are of interest, and it will be seen that the abortions in the positive herd had entirely subsided but the sterility figures are striking.

BREEDING EFFICIENCY RECORDS AT THE AGRICULTURAL SCHOOL MIQVE YISRAEL.

From October 1st, 1928, to September 30th, 1929.

	Infected Herd.	*Segregated Herd.*
Total number of herd	34	22
Normal calvings	23	22
Abortions	0	0
Conceived after 1 service	0	20
„ „ 2 services	3	1
„ „ 3 „	5	0
„ „ 4 „	4	1
„ „ 6 „	5	0
Still not pregnant after more than 6 services	9 } 19	
Sold as sterile	10 }	

Remarks.—The number 22, " Segregated Herd," included all cows and heifers which had calved before September 30th, 1929, this being the end of the year under observation.

Besides these there were at this date 11 heifers out of which 9 were in calf after one service, one after six services, and one which had been served nine times and was considered sterile.

An interesting feature in the records maintained at the School was a comparison of the weights of calves taken immediately after birth. These records showed that the average weight of calves born in the negative herd was 30 lbs. more per calf than the average weight of those born in the positive herd.

Requests for assistance were now becoming more insistent. In some cases these included demands for the import or supply of live vaccines. The import of such vaccine had been prohibited, as it was considered undesirable in view of the situation with regard to abortion that fresh strains should be introduced. A few controlled experiments with the use of vaccine in infected herds which were already under segregation control were made, but results were negligible and by this time the settlers were firmly convinced against vaccination procedures, and such requests have not been repeated.

The first settlement to apply for continuous testing, subject to an isolation agreement, was that of Beit Alfa, where an increasing number of abortions were taking place. A preliminary test had shown that nearly 50 per cent. of the cattle were positve. After 38 monthly tests the positive herd was disposed of, and only the negative herd now remains. The first onset of the disease was very acute, many abortions taking place after negative tests. (These cows all proved positive when tested immediately after the abortion.)

Following the example of Beit Alfa, other settlements evinced a desire to participate in control measures, and to meet this demand definite control plans and regulations were drawn up. Two types of plans were necessary: (*a*) Segregation Control, and (*b*) Prevention Control. Before undertaking either of these the settlements are requested to sign an agreement to comply with all the conditions laid down. These plans have now been in operation for four years, and 19 segregation and 26 prevention settlements,

comprising some 3,190 cattle, are now under control. Settlements under prevention control do not maintain positive herds and so are kept free from the disease. Of the 19 herds accepted under eradication control, no less than 14 have not had a positive case in their negative herds during the last six months and their transfer to prevention control merely awaits favourable opportunity for the disposal of their positive animals. The average period in which this has been accomplished was 20 months.

The negative herds only are tested, so that positive animals once removed from the herd are considered permanently infected and are not further tested. Occasional re-tests of positive herds have been made, and it has been found that the proportion of animals that have become negative, even after long periods, is very small.

The blood in each case is taken by Government veterinary officers and forwarded to the laboratory by the settlement. Test Record Sheets giving full particulars of each animal are also forwarded in triplicate so that complete records are maintained.

Under segregation control the usual period of testing is monthly, but this period is extended to three months when three or more consecutive negative tests have occurred. In some cases where infection is rapid, bi-monthly tests have been introduced, but this is not often necessary. Much depends on the circumstances and facilities for isolation in each case. In the case of prevention control six monthly tests are adopted, but are reduced to three months or less in special circumstances.

General Consideration.—A great deal of variation occurs in the types of stabling available at different settlements. At Beit Alfa, for example, the infected herd was located less than one hundred yards from, and at a higher level than, the stabling of the negative herd, towards which there was a natural slope. In other cases the distances between infected and negative portions of the herd are considerably less. Much depends on the particular circumstances and the energy and interest of the settlers. Isolation within the same stable has not been attempted, though it is not considered impossible where the conditions are sufficiently favourable. Where infected and non-infected herds are maintained, and even regardless of the distance between them, there is, however, always the possibility of re-infection of the negative herd by attendants or in some other manner. Such re-infection is occasional and is easily removed by regular testing.

Communal settlements in which the cattle are housed together, and which are able to provide for isolation, offer the best facilities, but individualist groups are also controlled if an agreement is undertaken to dispose of positive cattle. In these cases the separate owners each sign agreements and those who do not wish to do so are omitted from the tests. In some of these groups preliminary tests have shown but few positives, which have then been slaughtered, and in these cases the herds are easily placed on a

prevention basis. The value in Palestine of a prevention control cannot be over-estimated. With a small amount of trouble herds are insured against introduction of the disease, as it has always been found that the early removal of a few positives has protected against further spread. From such herds abortion-free cattle can be purchased to effect replacements in herds under eradication control and to meet increasing demands for the establishment of new herds and the requirements of small owners.

On two occasions a strongly positive animal was suddenly found in a herd which is under prevention control and had up to that time been entirely negative. In these cases the disease had probably been introduced by visitors from infected settlements. The disposal of the infected animal at once restored the herd to a clean state. Bulls are not considered an active source of infection to a negative herd and they are only tested at six months intervals.

Isolation of the calves from infected herds is provided for in the control plan. These are tested at six months of age and must pass two more negative tests before passing into the negative herd. It has quite frequently been the experience that calves remain positive up to 12 months old, and in some cases they never become negative. If they do not pass a negative test before they are 12 months old they are considered as permanently infected. Infection of a negative herd has been definitely traced to a calf with a low and " dying " titre of infection having been placed in close contact with susceptible cattle.

A considerable demand now exists from small owners for the test of cattle intended to be purchased. For this work a moderate charge is made. During the last year, 874 of these tests were performed with positive results in 123 cases. Contagious abortion is relatively much more frequent amongst small owners.

In the laboratory test four tubes are employed, commencing with a dilution of 1 :20. Positive cases are re-tested against various strains, as are also cases of reported actual abortion. All strains are examined, and plated and compared at intervals with known positive sera to ensure against loss of agglutinating power. Various other types of Brucella infecting animals and human beings are known to exist in Palestine, and at least one of these has been shown to be also capable of setting up bovine abortion (1), This strain, however, does not cause anything in the nature of a general outbreak and infected cows lose their titre quite rapidly. Serious infectious abortion has proved to be of a type identical with European strains of *Br. abortus*—a fact which has frequently been confirmed following isolation.

Although Brucellosis appears to be common, especially in sheep and goats, and unusual strains may be transmitted to cattle, it has not been found that native cattle offer any resistance to imported European strains. This was particularly noticeable in the case of the Beit Alfa settlement, where native cows were used

to grade up with Holland bulls. These cows were equally as susceptible as the half breds and the initial outbreak at this settlement was also very acute.

A considerable number of abortions occurring in different parts of the country are negative to all possible tests. These are usually sporadic cases, but a few instances have been observed in which several abortions have taken place in a non-infected herd within comparatively limited periods. These outbreaks subside in a few months. *Vibrio fetus* has on a few occasions been isolated, but does not appear to be more than an exceptional cause.

During the past twelve months some 20,000 agglutination tests in connection with control work have been carried out with positive results in 184 cases.

Doubtful reactions have occurred in 0·3 per cent. of the total number of tests carried out during the year. Re-tests of these are made, and the complement fixation test is also performed in a number of cases and is of considerable assistance in obtaining definite results.

The 19 infected herds under control comprised 1,725 cattle on initial testing. The following shows the total percentages of positives during 15 months, in periods of three months from the commencement of testing of each herd :—

Initial test	13·4	per cent.	positives
1st period	2·03	,,	,,
2nd period	3·42	,,	,,
3rd period	1·4	,,	,,
4th period	0·81	,,	,,
5th period	0·93	,,	,,

The following are some examples of the records of the testing of herds of different types :—

Table I shows the results of the first twelve tests at Beit Alfa. During the subsequent two years two fresh positives were discovered. The positive herd was then disposed of. Early in 1933 the disease was unfortunately re-introduced through the insufficient isolation of some newly purchased cows. Eight fresh positives were then found during the next few months, since when the herd has remained negative.

TABLE I.

No.	*Dated.*	*Positive.*	*Negative.*	*Total.*	*Positive. Per cent.*
1	24. 6.29	67	108	175	38·28
2	10.10.29	19	76	95	20
3	11.12.29	8	66	74	10.81
4	14. 1.30	6	64	70	7·37
5	10. 2.30	2	66	68	2.94
6	18. 3.30	2	69	71	2·81
7	24. 4.30	0	68	68	0
8	16. 6.30	1	74	75	1·33
9	20. 7.30	0	75	75	0
10	1. 9.30	0	75	75	0
11	10.10.30	0	75	75	0
12	1.12.30	3	72	75	3

Oiryat Anavim (Dilb) Settlement.—This outbreak has been one of the most difficult to control. Holland cows only are maintained and the outbreak was an acute one. The positive herd is still maintained at a distance of about 280 yards and at a lower level :—

TABLE II.

No.	*Date.*	*Positive.*	*Negative.*	*Total.*	*Positive. Per cent.*
1	23. 9.29	7	56	63	11·11
2	10.12.29	16	56	72	22·22
3	11. 3.30	11	59	70	15.71
4	8. 7.30	6	72	78	7·69
5	23.10.30	2	73	75	2·66
6	22. 1.31	8	70	78	10.25
7	27. 2.31	1	69	70	1·42
8	25. 3.31	6	68	74	8·10
9	13. 4.31	2	66	68	2·94
10	14. 5.31	4	73	77	5·19
11	10. 6.31	5	67	72	6·94
12	26. 6.31	7	78	85	8·23
13	10. 7.31	3	75	78	3·84
14	21. 7.31	0	71	71	0
15	6. 8.31	4	67	71	5·63
16	20. 8.31	3	47	50	6
17	4. 9.31	1	62	63	1·58
18	20. 9.31	1	54	55	1·81
19	9.10.31	0	56	56	0
20	26.10.31	2	68	70	2·85
21	6.11.31	0	53	53	0
22	19.11.31	1	67	68	1·47
23	4.12.31	1	67	68	1·47
24	18.12.31	0	67	67	0
25	5. 1.32	0	55	55	0
26	3. 2.32	1	66	67	1·49
27	24. 2.32	0	53	53	0
28	23. 3.32	0	66	66	0
29	22. 4.32	0	66	66	0
30	25. 5.32	0	65	65	0
31	23. 6.32	2	64	66	3·03
32	22. 7.32	0	70	70	0
33	26. 8.32	1	67	68	1·47
34	23. 9.32	0	70	70	0
35	28.10.32	1	71	72	1·38
36	23.11.32	0	70	70	0
37	23.12.32	0	74	74	0
38	24. 1.33	0	74	74	0
39	21. 2.33	0	74	74	0
40	25. 4.33	0	71	71	0
41	29. 5.33	0	67	67	0
42	26. 6.33	0	68	68	0
43	25. 7.33	0	69	69	0
44	28. 8.33	0	68	68	0
45	28. 9.33	0	67	67	0
46	30.10.33	1	68	69	1·44
47	4.12.33	0	68	68	0

Kfar Yehezkel Settlement.—This is a group of individual owners with separate stabling. The herd has mostly been tested at three months intervals:—

TABLE III.

No.	*Date.*	*Positive.*	*Negative.*	*Total.*	*Positive. Per cent.*
1	18.10.31	6	339	345	1·73
2	6.11.31	0	323	323	0
3	31.12.31	0	318	318	0
4	29. 1.32	0	306	306	0
5	22. 4.32	0	277	277	0
6	2. 8.32	0	266	266	0
7	15.11.32	0	248	248	0
8	12. 6.33	1	236	237	0·42
9	19. 7.33	0	238	238	0
10	8. 9.33	0	264	264	0
11	5.12.33	0	261	261	0

Kfar Gideon Settlement.—After removal of the initial reactors only one positive case has occurred. This is more or less the usual course of events in herds of this type:—

TABLE IV.

No.	*Date.*	*Positive.*	*Negative.*	*Total.*	*Positive. Per cent.*
1	4. 7.32	15	45	60	25
2	31. 1.33	0	14	14	0
3	1. 3.33	1	11	12	8·33
4	30. 3.33	0	10	10	0
5	25. 4.33	0	9	9	0
6	2. 6.33	0	8	8	0
7	2. 8.33	0	13	13	0
8	4. 9.33	0	12	12	0
9	3.12.33	0	10	10	0

Gesher Group.—This is an instance of a Group under prevention control in which a positive case has suddenly and somewhat unaccountably occurred. No further positives have been detected since its removal:—

TABLE V.

No.	*Date.*	*Positive.*	*Negative.*	*Total.*	*Positive. Per cent.*
1	19. 2.32	0	62	62	0
2	18. 3.32	0	62	62	0
3	19. 4.32	0	60	60	0
4	15. 7.32	0	58	58	0
5	28.10.32	1	57	58	1·72
6	9.11.32	0	58	58	0
7	25.12.32	0	54	54	0
8	23. 1.33	0	59	59	0
9	10. 4.33	0	54	54	0
10	5. 7.33	0	49	49	0
11	8.10.33	0	54	54	0

THE PRESENCE AND SIGNIFICANCE OF AGGLUTININS FOR SOME MEMBERS OF THE SALMONELLA GROUP OCCURRING IN THE SERA OF NORMAL ANIMALS*

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A previous communication (Lovell, 1932), dealt with the presence of agglutinins for the " H " heat-labile flagellar antigens of certain members of the Salmonella group which occur in the sera of healthy domesticated animals. The present work is concerned with the presence of agglutinins for both " H " and " O " antigens of certain members of the group occurring in such sera.

Technique.

Bacterial suspensions for agglutination purposes were prepared as follows :—

(*a*) For " H " flagellar antigens, formalinised broth cultures of the required phase which had been grown at 22° C. for 18 hours were used. The " H " suspension of *Bact. enteritidis* (the only monophasic strain used consistently) was prepared in a similar manner.

(*b*) " O " suspensions were prepared by cultivation on dry agar plates at 37° C. for 24 hours. The resultant growth was washed off with absolute alcohol and placed in a water bath at 56° C. for one to two hours. The suspensions were then centrifuged and the deposit was re-suspended in a small quantity of distilled water and, when required, made up in saline to an opacity equivalent to 1,000 million *Bact. coli* per c.c.

Both " H " and " O " suspensions were tested before use with homologous and heterologous sera.

The strains and suspensions used were as follows :—

(1) *Bact. ærtrycke*, strain " Glasgow," suspensions prepared were :—
- (*a*) group.
- (*b*) type.
- (*c*) " O."

(2) *Bact. newport*, strain " Shrewsbury," suspensions prepared were :—
- (*a*) type.
- (*b*) " O."

(3) *Bact. paratyphosum C* (Hirschfeld), strain " Sali mustapha," suspensions prepared were :—
- (*a*) type.
- (*b*) " O."

(4) *Bact. enteritidis*, strain " Wallasey," suspensions prepared were :—
- (*a*) " H."
- (*b*) " O."

*The data recorded in this paper were incorporated in a thesis approved for the Degree of Ph.D. (Bacteriology) in the University of London.

Agglutination experiments were performed by adding an equal quantity of the sera in varying dilutions to the various suspensions and incubating in a water bath at 55° C. Readings were taken after 2 hours and again after 24 hours incubation. With " H " antigens agglutination was of the coarse flocculating kind and complete in 2 hours, whilst with the " O " antigens agglutination was slower in formation and finely granular, and was never complete after two hours. The recordings are, therefore, for 24 hours, although rarely was there any difference in the 2-hours and 24-hours readings with the " H " antigens.

In the majority of cases samples of blood were taken when the animal was slaughtered for food and in no case was any abnormality found in the carcase. In a few cases blood was taken from living animals which appeared to be healthy. The dilutions used were 1/5 to 1/640 increasing by geometrical progression. If necessary then further dilutions were prepared. Unless stated, the serum was not treated after separation from the blood clot, but was kept in the cold room for from one to several days before testing.

Results of Experiments.

The following abbreviations are used :—

— = no agglutination at a dilution of 1/5.

A (g) = *Bact. ærtrycke*, group phase.
A (t) = *Bact. ærtrycke*, type phase.
A " O " = *Bact. ærtrycke*, " O " suspension.
N (t) = *Bact. newport*, type phase.
N " O " = *Bact. newport*, " O " suspension.
C (t) = *Bact. paratyphosum C*, type phase.
C " O " = *Bact. paratyphosum C*, " O " suspension.
E.G. (H) = *Bact. enteritidis*, " H " suspension.
E.G. " O " = *Bact. enteritidis*, " O " suspension.

TABLE I.

Showing Percentages of Samples of Sera Agglutinating various " H " Flagellar Suspensions at given Titres, Grouped.

Animal Species.	*Number Examined.*	*Titres.*	*A (g)*	*A (t)*	*N (t)*	*C (t)*	*E.G. (H)*
Swine ...	263	1/5 ; 1/10 ; 1/20	56·6	65·8	55·5	33·9	50·7
		1/40 and over	34·6	23·6	42·6	65·0	46·7
Cattle ...	163	1/5 ; 1/10 ; 1/20	73·0	33·7	43·5	26·3	10·4
		1/40 and over	18·5	66·3	56·5	72·4	78·9
Sheep ...	71	1/5 ; 1/10 ; 1/20	80·3	43·7	67·7	71·8	63·3
		1/40 and over	12·6	53·5	29·6	24·0	36·6
Horses ...	40	1/5 ; 1/10 ; 1/20	67·5	55·0	70·0	47·5	35·0
		1/40 and over	7·5	35·0	12·5	42·5	60·0
Rabbits ...	40	1/5 ; 1/10 ; 1/20	2·5	2·5	2·5	5·0	15.0
		1/40 and over	0	0	0	0	2.5

TABLE II.

SHOWING PERCENTAGE OF SAMPLES OF SERA AGGLUTINATING VARIOUS "O" SOMATIC SUSPENSIONS AT GIVEN TITRES, GROUPED.

Animal Species.	*Number Examined.*	*Titres.*	*A* "O"	*N* "O"	*C* "O"	*E.G.* "O"
Swine	263	1/5 ; 1/10	64·6	46·0	25·5	37·7
		1/20 and over	7·2	8·8	0·4	1·1
Cattle	163	1/5 ; 1/10	63·2	34·3	31·9	51·6
		1/20 and over	15·3	47·2	3·1	39·2
Sheep	71	1/5 ; 1/10	53·5	49·3	29·6	31·0
		1/20 and over	7·0	11·3	1·4	2·8
Horses ...	40	1/5 ; 1/10	57·5	55·0	40·0	62·5
		1/20 and over	30·0	25·0	0	22·5
Rabbits ...	40	1/5 ; 1/10	0	2·5	0	2·5
		1/20 and over	0	2·5	0	0

TABLE III.

GIVING THE MOST FREQUENT TITRE RECORDED IN THE SERIES OF OBSERVATIONS WITH EACH ANIMAL SPECIES.

Animal Species.	*A* (*g*)	*A* (*t*)	*A* "O" (*t*)	*N* (*t*)	*N* "O" (*t*)	*C* (*t*)	*C* "O" (*t*)	*E.G.* (*H*)	*E.G.* "O"
Swine	1/5	1/10	1/5	1/20	—	1/40	—	1/20	—
Cattle	1/10	1/40	1/10	1/40	1/20	1/40	—	1/80	1/10
Sheep	1/10	1/20 to 1/80	—	1/10	—	1/10	—	1/20	—
Horses	1/10	1/20	1/10	1/20	1/10	1/20	—	1/80	1/10
Rabbits	—	—	—	—	—	—	—	—	—

Tables I and II give the relevant data of the agglutination experiments, and record the number of samples of sera of each species examined, and the percentages agglutinating the various suspensions at given titres grouped. Owing to the consistently lower titres with "O" suspensions, the system of grouping is different in the two tables.

Table III gives the most frequent titre recorded in each of the animal species with each suspension.

Few comments are necessary, but certain details not easily apparent in the tables are dealt with.

Swine Sera.

The majority of the samples of swine sera agglutinated several suspensions, usually seven or eight. The "H" suspensions were in most cases agglutinated to a higher titre than the "O." The

commonest suspensions agglutinated were *Bact. paratyphosum C* (t), *Bact. newport* (t) and *Bact. enteritidis* (H), and the suspension least commonly agglutinated was *Bact. paratyphosum C* " O."

Cattle Sera.

Bact. ærtrycke (t), *Bact. newport* (t), *Bact. paratyphosum C* (t) and *Bact. enteritidis* (H) were the suspensions commonly agglutinated. *Bact. paratyphosum C* " O " is the suspension which was least commonly agglutinated, and then only to a low titre. All of the sera agglutinated the type phases of *Bact. ærtrycke* and *Bact. newport*. The " O " suspensions of *Bact. ærtrycke*, *Bact. newport* and *Bact. enteritidis* were agglutinated by most of the sera. It must be noted before passing that the majority of the samples were taken not from animals slaughtered for food but from milch cattle all of which were apparently healthy. As a large proportion came from one herd sampling was not at random.

Sheep Sera.

All these samples agglutinated *Bact. enteritidis* (H) to a titre of 1/5 or over, the most frequent titre being 1/20 and 97·2 per cent. agglutinated *Bact. ærtrycke* (type) to 1/5 or over. Two-thirds of the sera agglutinated the " O " suspensions of *Bact. ærtrycke* and *Bact. newport*, whilst only about one-third agglutinated the " O " suspensions of *Bact. paratyphosum C* and *Bact. enteritidis*.

Horse Sera.

Most of the samples were from old healthy horses slaughtered for animal food. They were all over ten years of age, the mean age being 15 years. The commonest suspension agglutinated was *Bact. enteritidis* (H), and the least common one *Bact. paratyphosum C* " O." Only 40 per cent. of the samples agglutinated this suspension to a titre of 1/5 or 1/10, the most frequent titre is, therefore, recorded as negative. The remaining suspensions were agglutinated by 75 per cent. or more of the samples.

Rabbit Sera.

For the sake of comparison, the results with 40 samples from laboratory rabbits are included.

Further comment is unnecessary, it being sufficient to record the comparative absence of normal agglutinins for the suspensions tested in laboratory rabbits. Of the 40 samples, 29 (72·5 per cent.) did not react with any suspension at a dilution of 1/5.

Influence of Age and Sex.

Age.

In order to ascertain whether any correlation existed between the age of the animal and the presence of normal agglutinins, the pigs were divided into 3 groups :—(*a*) three to four months old ; (*b*) five to six months old ; (*c*) seven to eight months old. Of the

263 examined, 45 fell in the first group, 54 in the second and 164 in the third.

It seemed clear from the analysis of the figures that with advancing age these normal antibodies show an increased frequency at the higher titres, whilst in younger pigs there is a corresponding decrease in those giving higher titres.

Another experiment supports this conclusion. Eight pigs belonging to one litter were bled at periods from 1 week old to 37 weeks, and their sera tested against the nine suspensions. The results are given in Table IV.

TABLE IV.

(Eight pigs.)

Absolute Numbers giving Agglutination at 1/10 or over with Sera of Eight Pigs belonging to One Litter, and Bled at Different Ages.

Age.	*A* (*g*)	*A* (*t*)	*A* "*O*"	*N* (*t*)	*N* "*O*"	*C* (*t*)	*C* "*O*"	*E.G.* (*H*)	*E.G.* "*O*"
1 week	3	2	0	0	0	0	0	3	0
3 weeks	0	0	0	0	0	0	0	0	0
6 „	0	0	0	0	0	0	0	0	0
11 „	1	0	0	0	0	0	0	0	0
20 „	0	0	0	1	2	2	0	3	0
25 „	0	2	0	4	0	3	0	4	0
37 „	3	3	3	8	5	3	1	5	4

The table is self-explanatory and affords additional evidence of the increased frequency of agglutinins for these suspensions with increasing age. The presence of agglutinins for the (H) suspensions of *Bact. ærtrycke* and *Bact. enteritidis* in the sera of 1-week-old pigs and their subsequent disappearance until several months of age suggests the passive transference of these agglutinins from the mother. Their subsequent reappearance would suggest their reaction to some stimulus, the nature of which is considered later.

A similar attempt was made to ascertain whether any such correlation existed with the cattle, but unfortunately a large proportion of the sera had been taken from milch cows all of which were adults. They, however, were divided into three groups :—

(*a*) Under three years old ; (*b*) from three to four years ; (*c*) four years old and over.

In the case of the " H " antigens there was a tendency for the sera of older cattle to react to a higher titre with greater frequency. Similarly, fewer older cattle failed to agglutinate or agglutinated suspensions to a low titre ; this was more marked with the extreme age groups. With the " O " suspensions there appeared to be no such relationship.

No attempt was made to classify according to age the sera of sheep, horses or rabbits.

Sex.

The samples of pig sera were classified according to sex, 139 being males and 124 females. Age was ignored, as the numbers

were too small to sub-divide by age and sex ; and, as the proportions in each age group were approximately the same, the grouping of all ages together was unlikely to obscure any sex differences. There was no evidence either with " H " or " O " suspensions of a significant difference between the male and female groups.

No attempt was made to classify the samples from the other species of animal according to sex.

Fractional Absorption of Sera.

Certain of the sera were absorbed with various suspensions and, although the time and temperature at which the absorption tests were carried out varied, the ratio of serum to suspension was kept constant, *viz.*, one part of pure serum to one-and-a-half parts of suspension. After a suitable time the mixture was centrifuged and the supernatant fluid, now in a dilution of $1/2\frac{1}{2}$, was tested for agglutinins. In the first four experiments (see Table V) the sera were only tested against " H " and " O " antigens. In the remainder they were tested against all. It will be noted that, unless specially stated, absorption was carried out with saline washings of whole bacteria, and if diphasic then both group and type phases were in eivdence. In certain cases the suspensions had been treated in order to annul the flagella and in those cases the symbol " O " is added.

The first two sera were tested for removal of " H " agglutinins alone, and there is some evidence of their specificity. In the first the monophasic (group) *Bact. suipestifer* reduces the group titre only from 1/640 to 1/160 ; *Bact. ærtrycke* reduces the group and eliminates its homologous type agglutinins only, whilst *Bact. enteritidis* removes its homologous agglutinins only. The second serum is acted on in a similar manner, diphasic bacteria reducing the group titre and eliminating or reducing considerably its homologous type titre. Owing to the high initial titres in these two sera, the possibility that these samples were from infected pigs must be considered, but it is unlikely that all the samples selected were from infected animals. The remaining sera show a similar reaction in regard to their specificity. It will be noticed that certain bacteria which share a similar antigen also have the power of absorbing the specific agglutinin. Serum Pig 242 had its type agglutinins for *Bact. newport* absorbed by *Bact. eastbourne*, which shares with that bacterium its type antigens (D_1, D_2). The strain " Moscow," which is very closely allied to *Bact. enteritidis*, shows a similar cross absorption in serum Horse 5. Agglutinins for the " O " antigens are similarly removed either by the homologous organism or by one sharing a similar antigen. " Potsdam," which shares the " O " antigen but not the " H " with *Bact. paratyphosum C*, is an example in serum Pig 249. *Bact. morbificans bovis*, which shares its " O " but not its type antigens with *Bact. newport*, behaves similarly with serum Pig 250. The two cattle sera examined support this antigenic specificity, it being remembered that *Bact. enteriditis* " Dublin "

TABLE No. V.

Absorption of Sera with various suspensions.

No.	Sera	A (g)	A (t)	AN "O"	AN (t)	N "O"	N (t)	C "O"	BG (H)	EA "O"
Pig 178	Unabsorbed control	640	40		5		10		10	
	Absorbed with Bact. suipestifer (g)	160	40		5		10		10	
	" " Bact. aertrycke	80	-		5		10		10	
	" " Bact. enteritidis	640	40		5		10		-	
Pig 180	Unabsorbed control	1280	160		10		10		5	
	Absorbed with Bact. suipestifer (g)	320	160		10		10		5	
	" " Bact. aertrycke	80	5		10		10		5	
	" " Bact. paratyphosum C	320	160		10		-		5	
Pig 209	Unabsorbed control			5		10		-		-
	Absorbed with Bact. aertrycke "O"			-		10		-		-
	" " Bact. newport "O"			-		-		-		-
	" " Bact. paratyphosum C "O"			10		10		-		-
Pig 220	Unabsorbed control			20		20		5		5
	Absorbed with Bact. aertrycke "O"			-		20		5		5
	" " Bact. abortus equi "O"			-		20		5		-
Pig 249	Unabsorbed control	20	5	-	20	10	160	10	80	-
	Absorbed with Bact. paratyphosum C	-	5	-	20	10	-	-	80	-
	" " "Potsdam"	10	5	-	20	5	80	-	80	-
Pig 250	Unabsorbed control	20	160	10	80	10	320	-	40	-
	Absorbed with Bact. aertrycke	-	-	-	80	10	320	-	40	-
	" " Bact. paratyphosum B.	-	160	-	40	10	160	-	40	-
	" " Bact. newport	-	160	10	-	-	160	-	40	-
	" " Bact.mortificans bovis	5	160	5	80	-	320	-	40	-
Pig 252	Unabsorbed control	10	10	10	20	10	80	-	160	10
	Absorbed with Bact. aertrycke	-	-	-	10	10	40	-	40	-
	" " Bact. newport	5	5	10	-	-	80	-	80	5
	" " Bact. enteritidis	10	5	5	20	5	80	-	-	-
Pig 242	Unabsorbed control	-	-	-	40	-	80	-	5	-
	Absorbed with Bact. paratyphosum C	-	-	-	40	-	-	-	-	-
	" " Bact. eastbourne	-	-	-	-	-	80	-	-	-
Cattle 87	Unabsorbed control			10		20		-		10
	Absorbed with Bact. enteritidis "O"			10		20		-		-
	" " Dublin "O"			10		20		-		-
	" " Bact. typhosum "O"			10		20		-		-
Cattle 88	Unabsorbed control			20		20		-		10
	Absorbed with Bact. aertrycke "O"			-		20		-		10
	" " Bact. paratyphosum B "O"			-		20		-		10
	"Stanley" "O"			-		10		-		10
Sheep 60	Unabsorbed control	10	20		20	10	20	5	40	-
	Absorbed with Bact. aertrycke "O"	5	5	-	20	10	20	-	40	-
	" " Bact. newport "O"	5	10	5	10	-	20	-	40	-
	" " Bact. paratyphosum C"O"	10	20	10	20	10	10	-	40	-
	" " Bact. enteritidis "O"	10	10	10	20	10	20	5	40	-
Sheep 61	Unabsorbed control	10	40	5	20	20	10	10	40	5
	Absorbed with Bact. aertrycke "O"	10	10	-	20	20	10	5	40	-
	" " Bact. newport. "O"	10	10	-	10	-	10	-	40	-
	" " Bact. paratyphosum C "O"	10	40	-	20	10	10	-	20	-
	" " Bact. enteritidis "O"	5	10	5	20	20	10	-	40	-
Horse 5	Unabsorbed control	-	20	5	-	10	20	-	80	10
	Absorbed with Bact. newport	-	20	5	-	-	10	-	80	10
	" " Bact. mortificans bovis	-	10	5	-	-	20	-	40	10
	" " Bact. enteritidis	-	10	5	-	10	20	-	-	-
	" " Bact. typhosum	-	20	5	-	10	20	-	80	-
	" " "Moscow"	-	10	5	-	10	20	-	-	-
Horse 14	Unabsorbed control	20	160	40	40	20	40	10	40	20
	Absorbed with Bact. aertrycke	-	-	-	20	20	40	10	40	10
	" " Bact. newport	-	160	40	-	-	40	-	40	40
	" " Bact.paratyphosum C	5	160	40	20	20	-	-	40	40
	" " Bact. enteritidis	10	80	20	40	20	40	10	-	-
	" " Bact. abortus equi	10	160	-	20	20	40	10	40	10
Horse 40	Unabsorbed control	10	10	10	20	10	20	5	20	10
	Absorbed with Bact. aertrycke	-	-	-	20	-	20	5	10	-
	" " Bact. newport	-	5	10	-	-	20	5	10	10
	" " Bact. paratyphosum C	-	5	10	20	5	-	-	10	10
	" " Bact. enteritidis	10	5	10	20	10	20	5	-	-

and *Bact. typhosum* all share the same " O " antigen (III), and the antigens I and II are shared by *Bact. ærtrycke*, *Bact. paratyphosum B* and " Stanley." One is, therefore, justified in maintaining that these normal agglutinins are specific when judged by absorption tests. Further, this specificity is not confined to agglutinins for whole bacteria but includes particular antigenic components of the bacteria. The absorption tests also support the existence of both flagellar and somatic agglutinins in normal sera, for in several instances it has been shown possible to remove the one without the other. One example only need be cited (Horse 5), where *Bact. typhosum* removes the agglutinins for the " O " but not for the " H " of *Bact. enteritidis*. There are one or two anomalous results, *e.g.*, Sheep 61, where the agglutinins for *Bact. ærtrycke* " O " were removed by *Bact. newport* " O " and *Bact. paratyphosum C* " O." As the unabsorbed sera only showed a titre of 1/5, it is felt that such a reduction is within the realm of experimental error ; in certain cases, however, it may be due to minor antigens. The existence of these was pointed out by White (1926) and have been omitted from later publications.

Effect of Absorption with Non-specific Substances.

Absorption with kieselguhr was tried with eight samples of sheep sera, all of which agglutinated several suspensions. To. 5 c.c. of serum was added 1 gramme of kieselguhr and the mixture was incubated at 37° C. for 1½ hours. The supernatant fluids, after centrifuging, were put up in parallel series with non-absorbed sera. The results showed no consistent difference between the sera before or after mixing with kieselguhr.

Effect of Heating Serum at 56° C. for Thirty Minutes.

Two pig sera and six sheep sera suspensions were heated in a water bath at 56° C. for 30 minutes and then tested in parallel series in order to ascertain whether this degree of heat would effect any reduction in titre. There was no observable reduction of titre in the heated samples and, therefore, under such conditions the agglutinating principle for both " H " and " O " antigens is heat-stable.

Agglutination of other Bacterial Suspensions.

Certain of the sera were also put up against suspensions of other bacteria. The results indicated that the serum of normal animals agglutinates a large number of bacterial suspensions some of which are widely distributed in nature and, as far as existing data go, are saprophytes. On the other hand, certain of the bacteria would appear to be pure parasites and it is unlikely that the agglutinins present could have been induced by the actual bacteria concerned. For example, *V. choleræ* was agglutinated to a titre of 1/10 by samples of sheep sera.

Attempted Culture of Salmonella Bacteria from Tissues of Normal Animals.

Attempts were made to isolate members of the Salmonella group from the tissues or intestinal contents of normal domesticated animals. All the animals examined possessed agglutinins for Salmonella bacteria in their blood serum.

Several techniques were adopted and consisted of cultivation of samples from pigs and sheep in plain broth, in broth with Salmonella agglutinating sera, and in broth containing brilliant green. Growths were subsequently plated on to McConkey's agar. In other cases large numbers of colonies were picked in duplicate from agar plates and subjected to Salmonella agglutinating serum.

Cultures were examined from 144 pigs and 71 sheep, but in no case was any member of the Salmonella group isolated. The following were the total numbers of samples examined :—

Animal.	*Fæces.*	*Mesenteric Glands.*	*Blood Clot.*	*Spleen.*	*Lungs.*
Swine (144)	18	130	—	4	5
Sheep (71)	—	21	24	50	—

Attempts to Stimulate the Production of Agglutinins by Heterologous Bacteria.

This work was suggested by the fact that specificity in the immunological sense may not correspond to specificity in the sense that it applies to systematic bacteriology. There are several instances on record where immunisation with a particular bacterium has induced agglutinins for a bacterium totally unrelated in the systematic sense. One of the earliest records is that of Avery, Heidelberger and Goebel (1925), who showed a serological and chemical association between the specific substances of a strain of Friedlander's bacillus and *Strept. pneumoniæ* Type II. Sugg and Neill (1929) present evidence of an immunological relationship between a variety of the yeast *Saccharomyces cerevisiæ* and *Strept. pneumoniæ* Type II, both by agglutination, and precipitation of crude filtrates. An association between the " O " antigens of *Bact. paratyphosum B* and allied Salmonella and certain strains of *Past. psuedotberculosis* has been pointed out by Schütze (1928) and confirmed by Kauffmann (1932). Considering these immunological cross relationships, it would not be surprising if a heterogeneous stimulation would supply the answer to the presence of the agglutinins which have been described in this paper.

Preliminary experiments showed the impossibility of stimulating Salmonella agglutinins in the serum of rabbits by inoculating filtered suspensions of fæces from normal pigs the sera of which possessed agglutinins for the Salmonella group. The failure in this case may have been due to insufficient dosage of the actual stimuli. Mixtures of cultures from the mesenteric glands of such pigs would on occasion, however, stimulate the production of agglutinins for Salmonella

bacteria to a low titre. Although it was found possible to repeat this observation using other rabbits, it was not found possible to produce such agglutinins by the injection of certain pure cultures of the bacteria concerned. This was found possible when dealing with cultures isolated from the fæces of normal pigs.

Two fæcal strains of coliform bacteria Numbers 242 (4) and 244 (ib) stimulated the production of Salmonella agglutinins in rabbits. In the one case agglutinins for the " O " suspensions of *Bact. newport* and *Bact. paratyphosum C* were produced whilst in the other case the rabbit serum agglutinated *Bact. newport* " O " and the " H " and " O " suspensions of *Bact. enteritidis*. Absorption tests were carried out, but as in no case did a titre of more than 1/160 appear they were somewhat difficult. Absorption of serum 242 (4) with the homologous bacteria reduced the titre for *Bact. newport* " O " from 1/160 to 1/40 and the titre for *Bact. paratyphosum C* " O " from 1/80 to 1/20. Absorption of serum 244 (ib) with the homologous bacteria reduced the titre of 1/10 for *Bact. enteritidis* (H) to below 1/5. Suspensions of *Bact. newport* " O " and *Bact. paratyphosum C* " O " removed agglutinins for themselves and for each other from a serum prepared against 242 (4) but had a slight effect only on the homologous titre, reducing the type of agglutination from a standard plus to a trace in the same dilution. Absorption of Salmonella sera with the fæcal cultures, however, gave negligible reductions.

It must be noted that the fæcal bacteria, 242 (4) and 244 (ib), were both coliform, producing acid and gas in dextrose, maltose, mannite and lactose ; salicin was positive with the latter but negative with the former. Neither strain acted on sucrose and both produced acid and clot in litmus milk.

The relationship between these bacteria present in the tissues and fæces of certain normal swine and Salmonella bacteria may only be slight. The relationship, however, appears to be sufficient to stimulate agglutinins for members of the Salmonella group and provides an explanation for their presence in the sera of normal swine.

General Discussion.

1. The presence of agglutinins for various bacteria in the sera of normal healthy animals has been noted by many authors (Uhlenhuth *et al* (1909), Wehrbein (1916), Savage (1918), Kinloch, Smith and Taylor (1926), and Gibson (1930)). The actual presence of normal agglutinins is not denied, but certain contradictory statements regarding the type of agglutinins have been made from time to time. Fishberg (1923), who examined the sera of normal humans for agglutinins for typhoid bacilli, states that normal agglutinins are stabilotropic (fine-flaking), that is, those which react with the " O " somatic antigens. Schiff (1922) has also concluded that normal agglutinins belong to the small-flaking stabilotropic type. Timmermann (1930) points out that Schiff contradicts himself, as he observes in one place

that agglutination was of a coarse nature and in another that it was fine and granular. Furthermore, agglutination occurred within 15 minutes, which is not characteristic of agglutination of " O " antigens. On the other hand, Breinl (1920) showed the presence of large- and small-flaking agglutinins in normal bovine serum for *Bact. typhosum*, *Bact. paratyphosum A* and *B* and *Bact. enteritidis*. Lovell (1932) showed the presence of agglutinins for the " H " heat-labile antigens of the Salmonella group in sera of normal swine, cattle, sheep and horses. Formalinised broth cultures were used as antigens and all agglutinations occurred rapidly and were of the large-flaking coarse type. The present investigation indicates the presence of both types of agglutinins in the sera of healthy animals. The " H " antigens were formalinised broth cultures which reacted rapidly in a coarse flocculating manner, whilst the " O " antigens were alcholised suspensions which reacted in a fine grnular manner and rather more slowly. Moreover, it has been pointed out above that it was possible by absorption to remove one type of agglutinin and to leave the other. The presence of both types of agglutinin in normal sera thus confirms the work of Breinl (1920), Timmermann (1930), Gibson (1931) and Jordan (1933). It is also in agreement with the recent work of Bosworth (1932-3), who in an attempt to facilitate the diagnosis of *Bact. abortus ovis* infection in sheep by the agglutination reaction, tested the agglutinin content of the sera of normal healthy sheep and of sheep from flocks in which infection with *Bact. abortus ovis* was known to exist. He found that agglutinins for both the " H " and " O " antigens of *Bact. abortus ovis* and of several antigenic components of other Salmonella bacteria are present in a fairly high proportion of normal sheep. The question of the specificity of normal agglutinins was studied by Gibson in his earlier work (1930) and he concluded that two factors were present, one a non-specific factor and the other specific. The former could be removed by saturating the serum with a finely divided absorbent. In his later paper (1931) he suggested that it is the large-flaking (H) agglutinins of normal serum which have two factors and that specificity depends chiefly on the specific factor of the " H " portion. He further suggested that the small-flaking " O " agglutinins possess affinities for unrelated organisms as he could not demonstrate the antigenic relationship between members of the Salmonella group with normal sera, as is possible with immune sera. There is some disagreement with the present findings, which showed that Salmonella agglutinins could not be absorbed from sheep sera with kieselguhr. Furthermore, in the majority of cases absorption with bacteria showed a definite specificity both with " H " and " O " agglutinins, and this specificity applies not only to the bacteria themselves but also to the actual antigens concerned. The results are of interest in view of the work of Mackie and Finkelstein (1931), Finkelstein (1933) and of Gordon and Carter (1932) and Gordon (1933) on the bactericidal power of normal serum. Whereas Mackie and his co-workers express the conviction from their experiments that absorption tests

demonstrate the high degree of specificity of the natural bactericida antibodies for particular bacteria, Gordon failed to establish the existence of specific bactericidal antibodies in normal sera. He states that "the loss of bactericidal power consequent upon absorption is never specific for the absorbing organism, but is always general." He bases this statement not only on experiments with normal rabbit and guinea-pig serum but also with normal pig serum, using the same strains of Salmonella bacteria as those which were used in the experiments recorded in this paper. His results are somewhat surprising in view of the specificity of the agglutinins for the various " O " antigens present in normal pig sera, for one would expect the normal bactericidal antibodies to go hand-in-hand with the normal " O " agglutinins.

2. The reports of attempted isolation of bacteria of the Salmonella group from the tissues or intestinal contents of normal healthy animals are also somewhat conflicting. A list of authors who have attempted to isolate members of the Salmonella group from normal animals is given, followed by certain criticisms of their findings.

Author.	*Animal.*	*Number Examined.*	*Number Positive.*	
Savage, 1906-7	swine	6	none	
	cattle	4	none	
1907-8	cattle	5	none	
	sheep	4	none	
	horses	3	none	
1918	swine	36	none	
	calves	10	none	
Uhlenhuth *et al*, 1908 ...	swine	600	51	8·5 per cent.
Seiffert, 1909	swine	60	2	3·3 „
Sobernheim, 1910	swine	51	3	5·8 „
	cattle	82	none	
	sheep	36	none	
	horses	50	none	
Schmidt, 1911...	swine	700	4 per cent.	only 1 per cent. can be accepted.
Aumann, 1911	swine	101	none	
	calves	10	none	
	cattle	48	none	
	sheep	41	none	
Bainbridge, 1911	swine	50	none	
Horn & Huber, 1912 ...	calves	100	none	
Huber, 1910	horses	100	none	
Fischer, 1915	cattle	105	none	
Meyer *et al*, 1916	calves	14	none	
Trawinski, 1917	swine	500	2	0·4 per cent.
Christiansen, 1917	cattle	34	none	
Jordan, 1918	swine	291	none	
Kinloch, Smith & Taylor, 1926	pigs	12	none	
	cattle	20	none	
	sheep	12	none	
Gheorghiu & Costin, 1927 ...	cattle	385	19	4·9 per cent.
Bermann, 1927	swine	78	3	3·8 „
	cattle	61	2	3·2 „
	sheep	6	1	
	horses	15	none	
Wolff, 1930	buffaloes	128	4	3·1 „
Lachenschmid, 1931	calves	550	none	
Lovell, 1932	swine	40	none	
Here recorded...	swine	144	none	
	sheep	71	none	

Savage (1906-7, 1907-8, 1918) is the chief opponent of the hypothesis of normal animals having members of the Salmonella group as part of their intestinal flora. He has reported on various occasions negative findings with the intestinal contents, spleen, gall bladder, bile and intestinal wall, of healthy animals and has stated that it would be a reasonable deduction that Salmonella bacteria are not found in the healthy intestines of the ordinary domesticated animals used for food. Uhlenhuth *et al* (1908) in the course of their investigations on swine fever examined the intestinal contents of 600 apparently healthy pigs. Bacteria which appeared by their pathogenicity, cultural biochemical and agglutination reactions, identical with those isolated from cases of swine fever were recovered 51 times (8·5 per cent.). Their biochemical tests were limited but all the strains were agglutinated by Salmonella anti-sera. Seiffert (1909) claims to have isolated two strains of Salmonella from the intestinal contents of 60 swine. Sobernheim (1910) isolated three strains from the tissues and intestinal contents of 51 swine, whilst Schmidt (1911) claims to have isolated *Bact. suipestifer* in about 4 per cent. of 700 samples of normal swine fæces. As two-thirds of his strains produced indol and only a quarter of them were agglutinated to titre by test sera, his figure should be 1 per cent. rather than 4 per cent. Other positive results have been recorded by Trawinski (1917) with swine, Gheorghiu and Costin (1927) with cattle, Bermann (1927) with swine, cattle and sheep, and Wolff (1930) with buffaloes killed in Dutch East Indies. Against this are the negative findings of Aumann (1911), Bainbridge (1911), Huber (1910), Horn and Huber (1912), Fischer (1915), Meyer *et al* (1916), Christiansen (1917), Jordan (1918), Kinloch Smith and Taylor (1926), Lachenschmid (1931), Lovell (1932) and those recorded in this paper.

As the list indicates, the findings would support the contention of Savage (1920), that the balance of evidence may be said to show conclusively that true Salmonella bacteria are not natural inhabitants of the animals used for food and that they are not found more frequently than can be accounted for on the supposition that their presence is due to an actual case of disease or the carrier state after infection. Jordan (1918), Bainbridge and O'Brien (1911) and Bainbridge (1912) also support this view.

3. The main question is the significance of the agglutinins which are present in the sera of a large proportion of domesticated animals. The obvious suggestion is that they are induced by previous infection with Salmonella bacteria. This is the suggestion put forward by Kinloch, Taylor and Smith (1926). Havens and Mayfield (1931) express a similar view regarding normal agglutinins in human beings. They suggest that the demonstration of antibodies for specific infection may be interpreted as the result of exposure to that infection at some time or other, and that the incidence of antibodies against a given infectious agent is one index of the prevalence of that infection in the region in which the study is made. One feels that this does not account for the presence of Salmonella agglutinins in domestic

animals. All the samples of swine and cattle sera and most of the sheep and horse sera gave positive reactions with one or more suspensions. One would, therefore, have to postulate infection of all those animals at one time or another with Salmonella bacteria. If that were so, then a proportion of those which had been examined for Salmonella bacteria should have been positive. This was not the case and it has been shown that the balance of evidence is against the hypothesis that normal domesticated animals carry Salmonella bacteria as part of their normal flora. Furthermore, one would have to postulate infection with several bacteria of the Salmonella group in order to agree to such a theory, for most of the sera agglutinated several suspensions which as far as we know are unrelated serologically. Infection with several varieites of Salmonella bacteria is unlikely to be the common occurrence.

In order to see whether the agglutinins for the various suspensions were distributed in accordance with the antigenic structure of common Salmonella pathogens of animals, some of the observations were examined statistically. One would expect associations between certain definite observations if the presence of these antibodies could be explained by previous infection with members of the Salmonella group. Probably the commonest Salmonella pathogen of swine in this country is the monophasic *Bact. suipestifer*. One would, therefore, expect an association between the ability to agglutinate *Bact. ærtrycke* (group) and *Bact. paratyphosum C* (O). But the ability to agglutinate any one suspension appeared to be related statistically to the ability to agglutinate other suspensions irrespective of any antigenic association between the two suspensions. There appeared to be no reasonable explanation to account for the association in the observations recorded, along the lines of stimulation by recognised Salmonella bacteria. The general evidence, therefore, appears to be against the hypothesis of these antibodies being due to infection with members of the Salmonella group.

The possibility of a correlation between the presence of agglutinins and natural immunity has been borne in mind. In this connection it is interesting to refer to the work of Bull and McKee (1921), who record experiments with the serum of normal chickens. The injection of such serum into mice and guinea-pigs conferred upon them a specific immunity to the various types of pneumococci. Kelley (1932) has recently shown that normal swine serum is capable of protecting mice against pneumococci and also of agglutinating both S and R pneumococci. In both cases the protective action of the serum was specific, that is, absorption of the serum with pneumococci of one type removes that particular protective substance alone. In this respect, Kelley likens swine serum to a polyvalent anti-pneumococcus serum produced by artificial immunisation, although it differs in other particulars. The agglutinins for pneumococci of one type also may be specifically absorbed, leaving those for pneumococci of other types unchanged. How chickens and pigs come to possess those antipneumococcal substances is puzzling.

One great difference between these records and those forming the subject of this paper is that whereas pneumococcal infection of chickens or pigs is unknown, infection of domesticated animals with members of the Salmonella group is quite common and there appears to be no association between the common Salmonella pathogens of the various species and the presence or absence of the corresponding agglutinins. Bürgi (1907) performed an interesting series of experiments in which he tested a small number of sera from guinea-pigs, men, rabbits, dogs, geese, sheep, goats, horses and cows against various bacterial suspensions. The sera tended to arrange themselves in groups, some of which agglutinated many of the tested suspensions whilst others agglutinated very few. The serum of the cow produced flocculation of all the suspensions tested, the serum of the guinea-pig very few, whilst the serum of man occupied an intermediate position. There seemed no reasonable explanation of these differences along the line of previous specific stimulation by natural infection and Bürgi adopted the view that the results obtained were an expression of the tendency of particular sera to agglutinate any bacterial suspension irrespective of its nature. Similar tests carried out with a mastic suspension tended to confirm this. In the series of papers by Mackie and his colleagues (Mackie and Finkelstein, 1928, 1930, 1931, 1932 ; Gibson 1930, 1931) a study of the various properties of normal serum has been made. These naturally occurring complement-fixing antibodies, agglutinins and bactericidal bodies which are present in normal sera are considered by them to be the precursors of those antibodies which arise in response to a specific stimulus. Conversely, it is suggested that immune antibodies have their precursors specifically differentiated in the serum of normal animals and that, in general, immune antibodies are not substances formed *de novo*. If one accepts the specificity of these natural antibodies for particular bacteria, whether bactericidal, complement-fixing, or agglutinating, then it would seem that the substances laid down in the animal's serum must be almost limitless.

It is difficult to correlate this distribution of various factors concerned in the properties of normal serum with some previously laid down mechanism, which arises without previous stimulation, and in the course of normal development. Hirszfeld (1926) found that the children of parents who differ in their Schick reaction and in blood grouping tend to resemble one parent in both these characters ; using genetic terminology, there would appear to be some linkage between the factor which determines antitoxin production and that which determines blood grouping. Whilst it is as well to remember that genetic and evolutionary factors may play their part in determining the antibody content of the serum of different animal species, it would appear to play a somewhat minor part, and it seems impossible to accept the view that these antibodies are purely evolutionary and arise irrespective of any specific stimulus.

There remains the theory that agglutinins may be due to stimulation by bacteria or substances which are unrelated from the systematic

point of view. Instances have already been recorded of the immunological and chemical association of apparently unrelated bacteria, and a certain amount of evidence has been adduced that coliform or other bacteria isolated from the intestine or mesenteric glands of normal swine have, when injected into rabbits, stimulated the production of agglutinins for members of the Salmonella group. It must be admitted that high titres were not obtained, neither were many suspensions agglutinated, but the results obtained certainly point in that direction for an explanation of this phenomenon. It is too early to theorise, but the suggestion is put forward that many of the bacteria which are normally present in the animal intestine may bear some very slight and immature relationship to the highly specific members of the Salmonella group, which appear to be the specialised bacteria with one or two components of their antigenic make-up highly developed.

For the moment it appears sufficient to state that agglutinins for both flagellar and somatic antigens of the Salmonella group are developed in the serum of normal pigs, sheep, cattle and horses. There is little or no evidence of their presence in normal rabbits. These antibodies are specific as judged by absorption tests ; they withstand 56° C. for 30 minutes and are not absorbed by a non-specific agent such as kieselguhr. There is no evidence in the present series of observations of any difference between the sexes as regards the distribution of these antibodies. There is evidence in some of the series of their increased frequency with advancing age and this is in accordance with previous work and the records of other authors. The significance of these normal antibodies and the explanation of their presence is thought most likely to be due to stimulation by bacteria which are normally present in the intestinal contents or tissues of the animals, but though not Salmonella bacteria have some slight antigenic association with them.

Summary.

(1) The sera of :—

263 normal healthy pigs,
163 normal healthy cattle,
71 normal healthy sheep,
40 normal healthy horses,
40 normal healthy rabbits,

have been tested for agglutinins for

Bact. ærtrycke (type, group and " O " suspensions).
Bact. newport (type and " O " suspensions).
Bact. paratyphosum C (type and " O " suspensions).
Bact. enteritidis (" H " and " O " suspensions).

The majority of the samples of serum from the pigs, cattle, sheep and horses agglutinated various suspensions, whilst the majority of the samples of rabbit sera failed to agglutinate any of the suspensions tested.

(2) There is a tendency for these normal antibodies to show an increased frequency at the higher titres with advancing age. This is evident in pigs, and with the " H " suspensions in cattle. The sheep examined were all of approximately the same age and all the horses were old so that no such conclusions can be drawn from those observations.

(3) Sex appears to bear no relationship to the frequency of normal agglutinins in pigs.

(4) As judged by absorption tests, these normal agglutinins are specific. This specificity is not confined to whole bacteria but is also apparent for the antigenic components of the bacteria, and supports the conclusion that agglutinins for both " H " and " O " antigens are present in normal sera.

(5) Saturation of sheep sera with kieselguhr did not reduce or eliminate Salmonella agglutinins.

(6) No reduction in titre was observed when samples of sera were heated at 56° C. for 30 minutes.

(7) Normal sera may agglutinate a large number of other bacterial suspensions, some of the bacteria being widely distributed in nature whilst others may be pure parasites.

(8) Samples of various tissues and intestinal contents of 144 pigs and 71 sheep were examined for Salmonella bacteria and in no case was a member of the group isolated.

(9) Injection of heterologous bacteria isolated from the tissues and intestinal contents of normal pigs have induced in rabbits Salmonella agglutinins to a low titre.

(10) The significance of these agglutinins is discussed, and it is thought most probable that their presence is due to stimulation by bacteria which are normally present in the intestinal contents or tissues of the animals, but are not members of the Salmonella group.

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EXCRETION OF *BR. ABORTUS* IN MILK.

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In an earlier report on contagious abortion in cattle (Report of Departmental Committee, 1905), the chief sources of infection were regarded as, firstly, the excretions of recently aborted cows and, secondly, infected bulls. At that time it seemed improbable that abortion bacilli were excreted in the milk of the affected cows and, some experimental observations were made to show that the bacilli could not be found in the milk a week after a large amount of culture had been injected up the teat. Again, there has been considerable controversy as to the natural mode of infection, but it would appear that infection can occur by ingestion, by passage of the organisms through the skin, mucous membranes of the eye and upper respiratory tract, and through the genital tract. (Departmental Report, 1905; Cotton and Bruck, 1931; Bang, 1932; King, 1932.) Recent reports by Fitch, Delez, and Boyd (1931), Gwatkin (1932), and Gaiger and Davies (1932), support the findings of previous workers in that, as a rule, *Br. abortus* is eliminated from the uterus and vagina within three or four weeks after abortion has taken place. It would appear, therefore, that lesions situated in tissues other than the uterus must maintain the infecting organism, and it would seem possible that, from the point of view of a carrier condition, the udder must exercise a more important part in the dissemination of infection than is generally recognised. Apparently the organism frequently becomes localised in the udder and neighbouring lymph glands and produces extensive changes in the form of interstitial mastitis.

Recent investigations on the excretion of *Br. abortus* in milk, and on the relationship of serum agglutinins to the presence of the organisms in the udder, have been reported by Klarin (1931), Tullberg (1931), Gilman (1931), Klimmer (1932), Cotton and Bruck (1932), Karsten (1932), and Proscholdt (1932). The findings of these workers, briefly summarised in the following table, show considerable agreement.

Observer.	*Titre of Serum.*	*Presence of Br. abortus in Milk.*
Tullberg	1/10 to 1/20	3 per cent. of cases.
	1/70 or under	19 per cent. of cases.
	1/100 or more	50 per cent. of cases.
Gilman	Under 1/80	Rare.
	1/80 or higher	59 per cent. of cases.
Klimmer	1/100 to 1/200	Rare.
	1/200 to 1/3,200	Variable.
	1/3,200 or higher	Practically all excretors.
Karsten	1/100	21 per cent. of cases.
	1/400 or more	64 per cent. of cases.
Proscholdt	1/100 or less	3 per cent. of cases.
	1/100	18 per cent. of cases.
	1/200	37 per cent. of cases.
	1/400	62 per cent. of cases.
	1/8,000	100 per cent. of cases.

There is some variation in the correlation between the presence of *Br. abortus* in the milk and agglutinin titres of serum. In general, cows with a serum titre of less than 1/100 rarely excrete *Br. abortus* in the milk, those with titres of 1/3,000 to 1/4,000 or higher practically all excrete *Br. abortus*, and in those with titres of 1/100 to 1/2,000 the results are variable. The definitive titres must vary with the technique of the agglutination test and with the sensitivity of the emulsion of *Br. abortus* employed in the tests by the various workers.

In previous investigations on undulant fever (Smith, 1932, 1934), it was found that raw milk must be the main factor in the dissemination of this disease to human beings. As it has already been found that some 28 per cent. of mixed milk samples contain *Br. abortus*, it was resolved to examine milk samples from individual cows, and the results are now presented.

Methods.

To determine the incidence of living *Br. abortus* in the milk of individual cows, animal inoculation methods have been used throughout. Thus, 50 c.c. of milk were centrifuged for 30 minutes at 3,500 r.p.m. Thereafter the supernatant cream and milk was discarded, the deposit was suspended in 6 c.c. of saline, and two guinea-pigs were inoculated in the groin with 3 c.c. of suspended deposit. One animal was killed after four weeks, and the second after eight weeks had elapsed. The blood was collected, and a serum agglutination test carried out. The spleen and part of the liver were removed with aseptic precautions, and placed in a sterile Petri dish, where they emulsified in saline, and a few drops were plated on liver agar plates. One plate was incubated at 37° C. in the normal atmosphere, another plate was incubated in a jar to which 10 per cent. of carbon dioxide was added, and both were examined after three days.

Agglutination tests were made with the whey. To obtain this, the milk was centrifuged, the cream discarded, a few drops of rennet added, and the mixture incubated at 37° C. In two or three hours the milk had coagulated firmly, and the clear whey was placed in Dreyer agglutination test tubes in dilutions ranging from 1/12½ to 1/3,200, one volume being 0·5 c.c. The suspension of *Br. abortus* used was that supplied by the Standards Laboratory at Oxford, 0·5 c.c. of the suspension being added to each tube of diluted whey. The tests were then incubated in a water bath for four hours at 55° C.; readings were then made, the tests were left at room temperature overnight, and a final reading made next morning. Agglutination tests were carried out in the same manner with the serum obtained from the killed guinea-pigs.

Results.

The results can be considered on the basis of two groupings: (1) the findings obtained from a herd of dairy cows which had been

vaccinated with living *Br. abortus* vaccine ; and (2) the results obtained from the examination of random milk samples from individual cows which had been sent to the Laboratory for examination for tubercle bacilli.

(1) *Findings in Herd of* 84 *Vaccinated Cows.*

The findings in this herd are given in Table I. From this it is seen that of the milk samples from 19 cows, which showed whey

TABLE I.

Comparison of Serum and Whey Agglutination Tests, and the Presence of *Br. Abortus* in the Milk as Shown by Guinea-Pig Inoculation.

	Agglutination :		*Guinea-Pig Inoculation of Milk* :			
Cow No.	*Blood Serum.*	*Whole Whey.*	*Pig.*	*Serum Agglutination.*	*Culture of Spleen and Liver.*	*Result.*
5	400	100	A	—	—	—
			B	—	—	
32	800	200	A	400	+	+
			B	800	+	
40	400	400	A	400	+	+
			B	3,200	+	
57	800	100	A	—	—	—
			B	—	—	
60	100	100	A	—	—	—
			B	—	—	
62	800	100	A	—	—	—
			B	—	—	
67	3,200	800	A	—	—	+
			B	200	+	
70	800	50	A	—	—	—
			B	—	—	
72	800	25	A	—	—	—
			B	—	—	
73	800	200	A	400	+	+
			B	800	+	
76	3,200	200	A	100	+	+
			B	200	+	
95	6,400	1,600	A	200	+	+
			B	—	—	
98	3,200	50	A	—	—	—
			B	—	—	
137	6,400	1,600	A	800	+	+
			B	1,600	+	
150	400	100	A	—	—	—
			B	—	—	
153	12,800	3,200	A	100	+	+
			B	1,600	+	
155	1,600	200	A	400	+	+
			B	1,600	+	
157	800	400	A	800	+	+
			B	3,200	+	
162	400	50	A	—	—	—
			B	—	—	

agglutinins in a dilution of 1/25 or more, only 10, or 11·9 per cent. were proved to contain *Br. abortus*, and the whey from all these agglutinated *Br. abortus* to a dilution of 1/200 or more.

Since all the animals had received living abortus vaccine one to two years previous to the present tests, serum and whey agglutinins were not an index of natural infection. In Table II are given the

TABLE II.

Agglutinins for *Br. Abortus* in the Sera of Cows Inoculated with 50 c.c. Living Vaccine 1 to 2 Years previous to Agglutination Test.

		No. showing Br. abortus in Milk.
No. of Sera negative	4	0
„ positive 1/25	8	0
„ „ 1/50	10	0
„ „ 1/100	18	0
„ „ 1/200	13	0
„ „ 1/400	16	1
„ „ 1/800	8	4
„ „ 1/1,600	1	1
„ „ 1/3,200	3	1
„ „ 1/6,400	2	2
„ „ 1/12,800	1	1
Total	84	10

i.e., 95·2 per cent. showed agglutinins in a dilution of 1/25 or more.

results of the blood serum agglutination tests. Of 31 of the cows with serum agglutinin titres of 1/400 or more, 10, or 32 per cent., excreted *Br. abortus* in their milk, and 9 out of 15, or 60 per cent. of those with serum titres of 1/800 or more, excreted the organism. It is interesting to note also that, despite vaccination and contact with infection, 4 out of the 84 animals failed to show agglutinins in the blood in a dilution of 1/25, while 95·2 per cent. gave positive agglutination tests in dilutions ranging from 1/25 to 1/12,800.

Furthermore, from 15 of the cows showing positive whey agglutination tests with milk from all quarters, samples from individual quarters were obtained, and whey agglutination tests carried out. These results are given in Table III. They show that there is some difference in the agglutinin titre of the milk from the various quarters. Furthermore, in all whey agglutination tests it has been found that the titre of agglutinins in the whey is often considerably less than the titre of the blood serum. Presumably, therefore, whey agglutinins are due to the presence of serum agglutinins in the milk, and the lower titre is due to dilution. In those cases in which the whey agglutinins are present the secretory apparatus has been damaged by infection—past or present—and this injury allows the serum agglutinins to percolate into the milk. There is no indication whatsoever that the whey agglutinins are formed locally

TABLE III.

WHEY AGGLUTINATION IN MILK SAMPLES FROM VARIOUS QUARTERS OF UDDER.

Cow No.	*Agglutination Titre of Serum.*	*Agglutination Titre of Whole Milk.*	*Quarter.*	*Whey Agglutination.*
32	800	200	1	200
			2	200
			3	200
			4	200
40	400	400	1	200
			2	200
			3	100
			4	400
57	800	100	1	200
			2	200
			3	25
			4	200
60	100	100	1	100
			2	0
			3	50
			4	100
62	800	100	1	25
			2	100
			3	50
			4	100
70	800	50	1	0
			2	50
			3	50
			4	0
72	800	25	1	25
			2	25
			3	25
			4	25
73	800	200	1	200
			2	200
			3	200
			4	800
95	6,400	1,600	1	6,400
			2	1,600
			3	1,600
			4	1,600
137	6,400	1,600	1	1,600
			2	1,600
			3	3,200
			4	3,200
150	400	100	1	100
			2	100
			3	100
			4	100
153	12,800	3,200	1	800
			2	800
155	1,600	200	1	200
			2	200
			3	100
			4	200
157	800	400	1	400
			2	800
			3	50
162	400	50	1	50
			2	50
			3	50
			4	0

Another interesting point arose in connection with the strains of *Br. abortus* which were isolated. Thus, two out of ten strains grew in the normal atmosphere without the addition of 10 per cent. carbon dioxide. These strains were probably established in the udder as a result of vaccination with a living *Br. abortus* organism, which culturally behaved in the same fashion.

(2) *Brucella abortus in Random Milk Samples from Individual Cows.*

In order to get some index of the prevalence of *Br. abortus* in the milk samples of individual cows, samples as sent to the Laboratory for tubercle bacilli were examined. The results obtained are set forth in Table IV. Thus, out of a total of 203 samples examined,

TABLE IV.

COMPARISON OF WHEY AGGLUTINATION TEST WITH THE PRESENCE OF *Br. Abortus* IN MILK AS SHOWN BY GUINEA-PIG INOCULATION.

Milk Samples:		*Guinea-Pig Inoculation:*			
No.	*Whey Agglutination.*	*Pig.*	*Serum Agglutination.*	*Culture of Spleen and Liver.*	*Result.*
1	—	A	1/200	+	+
		B	1/400	+	
2	1/200	A	—	—	—
		B	—	—	
3	1/800	A	—	—	+
		B	1/1,600	+	
4	1/100	A	—	—	—
		B	—	—	
5	1/50	A	—	—	—
		B	—	—	
6	1/50	A	—	—	—
		B	—	—	
7	1/1,600	A	1/800	+	+
		B	1/3,200	+	
8	1/50	A	—	—	—
		B	—	—	
9	1/100	A	—	—	—
		B	—	—	
10	1/50	A	1/100	+	+
		B	1/100	+	
11	1/100	A	—	—	—
		B	—	—	
12	1/50	A	—	—	—
		B	—	—	
13	1/3,200	A	1/400	+	+
		B	1/1,600	+	
14	1/400	A	1/100	+	+
		B	—	—	
15	1/200	A	—	—	+
		B	1/100	+	
16	1/3,200	A	1/100	+	+
		B	1/800	+	
17	1/200	A	1/200	+	+
		B	1/400	+	
18	1/25	A	—	—	—
		B	—	—	

TABLE IV. (*continued*).

Milk Samples: No.	*Whey Agglutination.*	*Pig.*	*Guinea-Pig Inoculation*: *Serum Agglutination.*	*Culture of Spleen and Liver.*	*Result.*
19	1/200	A	—	—	—
		B	—	—	
20	1/3,200	A	—	—	+
		B	1/400	+	
21	—	A	1/400	+	+
		B	1/800	+	
22	1/800	A	1/200	+	+
		B	1/1,600	+	
23	1/400	A	—	—	—
		B	—	—	
24	1/50	A	—	—	—
		B	—	—	
25	1/400	A	—	—	+
		B	1/400	+	
26	1/400	A	1/800	+	+
		B	1/1,600	+	
27	1/25	A	—	—	+
		B	1/200	+	
28	1/800	A	1/400	+	+
		B	1/3,200	+	
29	1/400	A	1/400	+	+
		B	1/1,600	+	
30	1/400	A	—	—	+
		B	1/400	+	
31	1/200	A	—	—	+
		B	1/800	+	
32	1/100	A	1/200	+	+
		B	1/400	+	
33	1/400	A	—	—	+
		B	1/400	+	
34	—	A	—	—	+
		B	1/200	+	
35	1/400	A	1/200	+	+
		B	—	—	

Br. abortus was recovered by guinea-pig inoculation from 23, or 11·3 per cent., a figure comparable with that obtained from the herd of vaccinated cows. Here again whey agglutinins in a dilution of 1/200 or more indicated that *Br. abortus* would probably be found by animal inoculation. In three instances, however, no whey agglutinins were demonstrated, and yet guinea-pig inoculation showed *Br. abortus*; while in two instances where the whey agglutinin titres ranged from 1/25 to 1/100 the animal result was positive; and on one occasion when the whey titre was 1/400 animal inoculation failed to show the presence of *Br. abortus*. Again, cultural examination showed that 3 out of the 23 strains grew in a normal atmosphere, whereas the others required the addition of 10 per cent. carbon dioxide before growth occurred. All strains were agglutinated to the full titre of a *Br. abortus* agglutinating serum, and all absorbed the agglutinins.

Discussion.

The results clearly show that a considerable number of cows are continuously excreting *Br. abortus* in the milk. In the present investigation 33, or 11·5 per cent., of milk samples out of a total of 287 were found to contain this organism. The majority of workers are agreed that the most effective method of isolating *Br. abortus* from milk is by guinea-pig inoculation. The present results confirm previous work in indicating that two guinea-pigs should be employed for this test, as on occasion the infecting dose of organisms is apparently not sufficiently great to overcome a certain amount of natural immunity in some animals. This is evidenced by the fact that one animal may show a positive serum agglutination and a positive spleen and liver culture, whereas in the other animal the findings may be completely negative.

Again, the agglutinin titre of the cow's serum bears a relationship to the excretion of *Br. abortus* ; thus the higher the titre the greater is the possibility of the animal having an infection of the udder. A similar connection can be established between whey agglutinins and the presence of the organism, as a whey agglutination titre of 1/200 or more is certainly significant of active excretion, although on occasions milk showing no agglutinins will contain the organism.

The results of various workers can be examined only in a comparative manner, and the significant titres for serum and whey agglutination must depend on the technique of the test and on the agglutinability of the suspension of *Br. abortus*. The agglutination method employed in this series of observations produces high titres, since the emulsion is very sensitive and incubation is carried out at 55° C. in the water bath. If, however, a standard technique and a standard antigen are employed by workers throughout the country, then the results will be comparable. At any rate, the higher the agglutinin titre of the serum and whey for *Br. abortus*, the greater is the possibility of an animal being an active carrier. It seems that in view of the serious economic loss to farmers through contagious abortion, and in view of the fresh interest that has been aroused by the fact that human beings can contract infection, the time has come to investigate every aspect of this problem. At present eradication schemes are based on positive agglutination tests, but by analogy with other animal and human infections it is evident that such a test must eliminate all animals which have been infected at some time or another and yet are not necessarily carriers of the organism. It is essential, therefore, that the carrier problem in contagious abortion should be thoroughly investigated to ascertain what secretions besides milk contain *Br. abortus*.

Conclusions.

1. In a herd of 84 cows vaccinated with a living culture of *Br. abortus*, 10, or 11·9 per cent., were excreting *Br. abortus* in their milk.

2. From 203 random samples of milk from individual cows, 23, or 11·3 per cent., were found to contain *Br. abortus*.

3. From a total of 287 samples of milk from individual cows, 33, or 11·5 per cent., were found to contain *Br. abortus*.

4. The greater the serum agglutinin titre for *Br. abortus*, the greater is the likelihood of the animal excreting *Br. abortus* in the milk.

5. By the agglutination method employed, a whey agglutinin titre of 1/200 or more was significant of excretion of *Br. abortus*.

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A SURVEY FOR THE PRESENCE OF BRUCELLA AGGLUTININS IN PIGS' BLOOD IN GREAT BRITAIN.

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The presence of *Brucella suis* infection in a country is of considerable importance both from the Public Health aspect and because of its potential economic effect upon pig breeding.

It has been established that porcine strains of brucella are more pathogenic than bovine strains for human beings and these strains have frequently been isolated during recent times from the udders of cows.

The porcine disease has been reported from many parts of Europe (Hungary, Russia, Germany, Denmark, Switzerland and France), and it is widely distributed over the North-American continent.

There is no record of its existence in Great Britain and there are few reports of illness of pigs suggestive of the disease.

The absence of reports of abortion occurring in sows is not, however, sufficient in itself to rule out the possibility of the infection being present on a small scale.

It is generally recognised that brucella infection in pigs is not characterised by abortion to anything like the same extent as it is in bovines ; the birth of weak or undeveloped dead pigs, followed occasionally by lameness and to a variable extent by temporary sterility, appears to be the principal feature of the porcine infection. Instances of abortion " storms," similar to those which occur among cattle, have occasionally been reported.

In the absence of information regarding the disease in Great Britain, it was decided to submit to the agglutination test a number of samples of pigs' blood obtained from different parts of the country.

Surveys based upon the agglutination test to ascertain the incidence of brucella infection of pigs have been carried out in Europe and in many parts of America, and sufficient data have been accumulated to confirm the accuracy of the test for this purpose.

Weeter (1923) tested the blood of 435 sows and 64 (14·7 per cent.) reacted in dilutions of 1/50 or higher, and 39 (9 per cent.) reacted in a dilution of 1/100 or higher.

Graham and Tunnicliff (1927) tested blood samples collected in a Chicago packing house. Out of 1,011 sows 5·6 per cent. gave positive reactions, of 975 gilts tested 4·41 per cent. were positive, and of 1,034 bloods from fat pigs 3·38 per cent. were positive. The agglutination titre accepted as indicating infection is not stated.

Boak and Carpenter (1930) tested 4,014 pig bloods, obtained from eight different states, and 1·54 per cent. reacted in a dilution of 1/60 or higher.

Gwatkin (1931) in Canada tested 500 sows and three gave positive reactions (1/100) and four gave suspicious reactions (1/50).

Thompsen (1931) records that the first case of *Br. abortus* infection of pigs in Denmark was recognised in 1929 and that by 1931 the infection was present on more than 200 farms.

Johnson and Huddleson (1931) tested 15 herds in Michigan and found the disease to be prevalent, reactors being found in every herd. The degree of infection, based upon a titre of 1/50 or higher, varied up to 23 per cent.

Murray, McNutt and Purwin (1931) in Iowa tested blood samples from 748 sows and 4·2 per cent. reacted, but on farms where cases of undulant fever had occurred the porcine infection averaged 23·5 per cent.

Starr (1933) in Virginia tested 1,316 samples and 42 (3·2 per cent.) gave a positive reaction in a dilution of 1/100 or higher, and 313 (23·7 per cent.) gave suspicious reactions.

Huddleson, Johnson and Hamann (1933) examined the blood of 3,975 pigs slaughtered at two packing houses in Michigan. The examinations were made by means of the rapid whole-blood agglutination test, using a single dilution capable of detecting agglutinins in a dilution of 1/50 or over. 308 pigs (7·74 per cent.) reacted and from 41 (13·31 per cent.) of these *Br. suis* was isolated from one or more tissues.

Hayes (1934) has examined the results of the various surveys carried out in the United States and considers that in those states where pig breeding is extensively practised about 5 per cent. of pigs are positive to the agglutination test for brucella. He points out that as many as 20 per cent. of pigs react in dilutions of 1/25 to 1/50, and studies made on these pigs indicated that many were carriers, and probably at some previous test had reacted in higher dilutions.

Origin of *Brucella suis*.

The origin of porcine brucella is a problem of considerable importance and interest. It was at one time considered probable that pigs contracted the disease from contact with infected cattle, and there appeared to be some presumptive evidence in support of that view.

Hayes (1922) believed that pigs derived infection from cattle, probably through the ingestion of fœtal membranes, aborted fœtuses, vaginal discharges, or milk containing *Br. abortus*.

Carpenter and Baker (1927) were of the same opinion and stated that, as pigs were less susceptible than cattle to brucella infection, only the most virulent bovine strains were capable of infecting them.

Further knowledge of the porcine infection has been acquired with the passage of time, but no evidence has been adduced to support the contention that *Br. suis* originates from bovine strains

of brucella, and it is now generally believed that there is seldom any connection between the disease in bovines and in pigs.

Further evidence in support of this view is to be found in the apparent absence of the porcine infection in Great Britain, although there is a high and widespread incidence of the bovine disease, and there is ample opportunity on many farms for pigs to contract the disease from direct contact with infected cattle.

Cotton (1930) pointed out that the question of the relationship between the bovine and porcine infections has not yet been settled, but that it would appear from the evidence available that the two infections are not inter-communicable to any great extent by natural means, though they are by inoculation.

There would appear, however, to be considerable danger of cattle becoming infected with *Br. suis* and there are a considerable number of records of this organism having been isolated from the udders of cows.

Gilman, Milks and Birch (1934) have recently passaged *Br. abortus* strains through a series of sows to ascertain if this procedure would result in the bovine strains assuming the characteristics of porcine strains as judged by the dye tolerance tests. It did not, however, bring about any appreciable change in the bovine strains.

In a general way, the three types, *abortus*, *suis* and *melitensis*, appear to be specific for their respective natural hosts, cattle, pigs and goats.

The Agglutination Test.

Some observers have suggested that brucella agglutinins do not persist for any considerable time in pigs' blood. It is known that pigs do not usually remain permanently infected as is the case with the majority of infected cattle, but there is ample proof that agglutinins persist in detectable amounts for a sufficient period to render a survey based on their presence of practical value.

Connaway (1922) was of the opinion that naturally infected sows generally remain premanent reactors to the agglutination test.

Hayes (1922) carried out monthly agglutination tests of naturally infected pigs and observed that in the majority of cases there was little fluctuation in the titres over a period of one year, but after that period the titres gradually declined.

Howarth and Hayes (1931) found considerable fluctuation in the titres of reacting sows ; the titres appeared to decline at the time of farrowing and to rise afterwards to the original level.

Johnson and Huddleson (1931) brought forward experimental data proving that brucella agglutinins in pigs' blood disappear earlier than in bovine blood. They considered that in most instances the organisms do not persist in the body of the pig for more than from three to five months.

Hayes (1934), who has had a considerable experience of the

agglutination test for the diagnosis of brucella infection in pigs, regards it as being equally as reliable for the infection in cattle. He considers it probable that a higher percentage of infected pigs react in the low dilutions than in the case of cattle and that the number of suspicious cases among pigs is also relatively higher.

Technique of Test.

The antigen consisted of a 72-hour-old broth culture of *Br. abortus*, the density of which was increased by the addition of a small amount of a 48-hour-old washed agar growth in order to match its capacity with Brown's No. 1 tube.

Dilutions were set up in 1/10, 1/25, 1/50, 1/100, 1/200, and incubated for about 20 hours at 37° C.

All sera giving positive reactions in 1/25 or over were re-tested with *Br. abortus* antigen obtained from the Standards Laboratory, Oxford, and also by the rapid plate method. There was little difference between the titres obtained with our antigen and with the Oxford antigen. The plate method always confirmed the diagnosis, but occasionally there was some variation in the titre end-points as shown by this method.

Many of the pigs' bloods showed a heavy, non-specific, flocculent precipitate.

On the remote chance that brucella organisms might be present in the positive blood samples, the blood clots were broken down, mixed with the serum and injected intraperitoneally and intramuscularly in 5 c.c. doses into guinea-pigs.

The guinea-pigs were killed about the 44th day after inoculation, agglutination tests were carried out with their blood sera, and cultures made from the spleen on liver agar medium, but in every case with negative result.

Interpretation of the Test.

There appears to be some difference of opinion as to the agglutination titre that should be accepted as indicating brucella infection in pigs.

Fitch pointed out that the Abortion Committee of the National Research Committee considered that: "reactions of 1/100 or higher should be regarded as positive and reactions of 1/50 upwards as suspicious."

Carpenter believes that the agglutination of *Br. abortus* in a dilution of 1/25 by porcine serum is not a specific reaction.

Johnson and Huddleson (1931) regard positive reactions in dilutions of 1/25 as signifying exposure to brucella infection and this view is based upon the results obtained from the bacteriological examination of reacting pigs.

Hayes (1934) considers that all pigs of breeding age which show a positive reaction in a dilution of 1/50 with an antigen concentrated

to a Gates 4·0 or McFarland 2·5 should be considered highly suspicious and that they are probably carriers.

He records that the bacteriological examination of 67 reacting pigs demonstrated the *Brucella suis* in about 2 per cent. of those slaughtered when the agglutination titre was not above 1/50, and in 75 per cent. of those with titres of 1/100 or higher.

An important point to which consideration should be given when interpreting the results of tests is whether or not a herd is infected. A positive reaction in a dilution of 1/25 is, in a proved infected herd, of significance and obviously cannot be safely ignored.

Tests of Pigs' Blood.

Through the courtesy of the veterinary staffs of firms of bacon curers and of municipal veterinary officers, arrangements were made to obtain blood samples from sows at the time of slaughter.

The blood was taken only from sows that had been bred from, as it was considered that if porcine brucella infection existed in the country it would most likely be found in this type of animal.

An additional reason for procuring the blood from adult animals was that there is strong evidence, young pigs have considerable resistance to brucella infection, similar to that which occurs in calves, and that this resistance persists until sexual maturity is reached.

Howarth and Hayes (1931) have shown that the majority of young pigs, even though they be the progeny of brucella-infected dams and are kept in close contact with them, give negative reactions to the agglutination test up to at least four months of age.

In this survey, samples of blood from 10,474 sows have been submitted to the agglutination test for the presence of brucella agglutinins. The bloods were obtained from 14 different sources, and according to the information supplied the pigs originated from various parts of England, Scotland, Wales and Ireland, so that they can reasonably be regarded as being representative of the pig population of Great Britain.

Table I.
Results of Tests.

Number of Bloods examined.	*Number Negative.*	*Positive.*		
		1/25	1/50	1/100
10,474	10,449	4	17	4

Discussion.

It will be seen from Table I that four bloods gave positive reactions in a dilution of 1/25, seventeen bloods positive reactions in a dilution of 1/50, and four bloods positive reactions in a dilution of 1/100.

In the absence of information regarding the occurrence of the disease in Great Britain, it is proposed to dismiss from further consideration the reactions that occurred in the 1/25 and in the 1/50 dilutions.

The four blood samples that gave positive reactions in the 1/100 dilution must, however, be considered, and it is difficult to see how any other interpretation can reasonably be placed on these than that they signify existing or recent brucella infection. To regard these reactions as being of no significance would be to take an unduly optimistic view of the situation as revealed by this survey.

It is recognised, however, that until such time as the *Br. suis* is isolated from a pig bred in this country the question of the existence here of brucella infection in this species cannot be decided.

It is impossible in the present state of our knowledge to predict the future course and importance of *Br. suis* infection. Its further dissemination in all countries in which pig breeding is extensively practised seems reasonably certain, but as the disease appears to be self-limiting it is improbable that it will attain to similar proportions or exercise such serious adverse economic effect on the pig industry that the *Br. abortus* does on the cattle industry.

It is clear that there is little, if any, *Br. suis* infection in Great Britain, but how long this fortunate situation will remain cannot be foreseen.

Charles Nicolle (1930) termed undulant fever a *maladie d'avenir*, which had originated in the goat and then passed to man.

Hayes (1934) referring to *Br. suis* infection in America, states :—

> " There is no doubt but that it is slowly spreading over this country, even though its spectacular effects may not be so evident as in the disease in cattle and the monetary loss not so great to the swine industry. However, this is counterbalanced certainly by the danger to human beings and by its effects upon the programmes for controlling Bang's disease in cattle that are now in progress."

In view of the proved pathogenicity of *Br. suis* for man its possible introduction and dissemination in this country is a matter that demands serious consideration, and careful watch should be kept for infections of breeding sows suggestive of the disease.

On account of the insidious nature of the infection and the low abortion rate usually associated with it, it may easily obtain a foothold before being detected if serological tests are not carried out in all suspicious cases.

Conclusion.

Samples of blood from 10,474 sows have been submitted to the agglutination test for the presence of brucella agglutinins.

Four bloods gave positive reactions in dilutions of 1/25, seventeen bloods positive reactions in dilutions of 1/50, and four bloods positive reactions in dilutions of 1/100.

Positive reactions in dilutions of 1/100 are generally regarded as indicating existing or recent infection with *Brucella suis*.

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ECTOPIA CORDIS IN THE CALF.

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Ectopia cordis is apparently not a very rare condition, for a number of cases have been reported as occurring in both man and the lower animals.

Sidney Jones[1] classifies the varieties as follows :—

A. *Internal*—where displacement of the heart occurs within the body,

1. Where displacement of the heart occurs without any other deformity.

2. Where an imperfect diaphragm allows the heart to enter the abdominal cavity.

B. *External*—where displacement occurs without the body,

1. Cervical—when the heart is situated in front of the neck, either immediately above the thorax or in connection with the under part of the head.

2. Abdominal—when the heart forms a hernial protrusion with the abdominal viscera.

3. Thoracic—when the heart protrudes outside the chest.

The present case was of the cervical variety, and the subject was a white Shorthorn heifer calf. The animal died 60 hours after birth. I am indebted to Mr. O. Stinson, Veterinary Surgeon, Appleby, for sending me the carcase together with the photograph reproduced in Fig. 1.

Cervical ectopia would appear to be the most common variety seen among domestic animals. Gurlt[2] and Walter[3] have noted its occurrence in lambs, I. G. St. Hilaire[4] reports seeing cases in dogs, and several observers have reported the condition in calves. Complete accounts of the anatomy of the parts concerned, however, appear to be wanting. The reports of both Walley[5] and Morgan[6] are accompanied by good photographs which lead one to believe that the condition they encountered was not unlike that to be described. But only Waterston[7], who gives a full description of the heart as received by him, and Van de Pas[8], whose report includes a brief account of the heart and adjacent vessels, appear to have made detailed dissections. (Van de Pas' case concerned one of two animals on which Houssay and Giusti[9] had made cardiographic studies previously).

The age to which animals thus affected live is variable. Lothian[10] reports a case the subject of which lived for a little over a month, whilst Ison[11] and Harbaugh[12] each saw an animal which lived for two years.

A superficial examination of the carcase of the calf under discussion revealed the fact that the heart occupied a fixed position in the ventral

part of the neck, giving rise to a large swelling extending forwards from the entrance to the chest to within a short distance of the mandibular space. The swelling appeared slightly more prominent on the left than on the right side. This fixation of the heart may be compared with Ison's case where the heart was quite movable and could be forced back into the thorax. "It was," he says, "outside the pericardium, the latter being very elongated and narrow, extending from the frontal region to the diaphragm." Handley[13], too, mentions a case where the heart was free, and immediately

FIG I.
White Shorthorn calf. Note swelling in ventral part of neck due to the ectopic heart

after birth "could be moved up the neck as far as the angle of the jaw, but at two weeks of age adhesions had formed and the heart was firmly attached to the skin and surrounding tissues."

The skin of the region presented no peculiarity. It was thickest over the anterior part of the swelling. After its removal a thin layer of panniculus was noted. This was best developed in the anterior part of the neck.

The pre-tracheal muscles were ill-defined. Extending from the sternum to the mandibular space, they formed a thin sheet of muscle which covered the ventral and lateral parts of the pericardium

lying beneath. The sterno-cephalici were generally normal except at their origin, which was aponeurotic, but the sterno-thyro-hyoidei appeared to arise from the medial edges of the sterno-cephalici about half-way up the neck. The other muscles of the neck showed little deviation from the normal.

The thorax was shorter than usual, especially ventrally, and its entrance from the neck was wide. The sternum was peculiar in its anterior part. It was exceptionally broad here and the centres of ossification lay further than usual from the middle line. Between them the upper surface of the sternum was widely depressed, and just behind the depression the sterno-pericardiac ligaments were attached. The ribs were typical in number, but the cartilages of the first few curved outwards, thus further widening the space between these ribs. The pectoral and other muscles of the chest wall were normal. The extensions of the pleural sacs beyond the first ribs were prominent, the right sac extending for a distance of about 4 inches over the lateral surface of the pericardium under cover of the sterno-cephalicus, and the left projecting similarly for a distance of about 2 inches. The endothoracic fascia was very strong, and its extension beyond the chest, *i.e.*, where it covered the pleural *culs-de-sac*, blended with the aponeurotic origins of the sterno-cephalici and sterno-thyro-hyoidei. The lungs showed considerable areas of atelectasis. They occupied the greater part of the thoracic cavity, since, excepting the base of the heart, all the structures of the normal anterior mediastinum lay outside the cavity. The right lung showed the usual number of lobes, the apical being subdivided by a fissure extending almost to the upper border of the organ. There was no notch on the inferior border. The left lung possessed only two lobes, the apical and cardiac being entirely fused. As in the case of the right lung, nothing corresponding to a cardiac notch was present.

The pericardium was complete, and, like the heart, very elongated from before to behind. The base lay a little behind the first pair of ribs, whilst the apex was opposite the fifth ring of the trachea. The sac was related ventrally to the two sterno-cephalici and sterno-thyro-hyoidei muscles. Laterally, too, these muscles covered the sac except near the thorax, where the extensions of the pleural sacs intervened. A layer of loose, fat-laden fascia lay between these muscles and the pericardium. Dorsally, a denser layer of fascia, part of the cervical fascia, separated the sac from the overlying thymus gland. Apart from its being supported by the pre-tracheal muscles, the pericardium was suspended on either side by strong sheets of this fascia, which ran from its supero-lateral aspects upwards beneath the scaleni to blend with the fascia lying beneath the longus colli and over the thymus gland. These sheets were irregular in structure and at the entrance to the chest they blended with the endothoracic fascia. From the apex of the pericardium detached bands of the fascia continued forwards on either side, blending with the insertion of the sterno-mandibularis and joining the fascia covering the parotid and submaxillary glands. These bands extended

forwards from each side of the apex of the sac, with the result that the interior of the sac showed at its apex two depressions separated in the middle by a ridge. Cord-like thickenings, the sterno-pericardiac ligaments, also ran from the base backwards, to end on the floor of the thorax at the level of the third chondro-sternal joints.

The heart was elongated antero-posteriorly, and compressed from above to below. The base lay just within the thorax, and the apex was 2 inches behind the level of the cricoid cartilage. The circum-

FIG. 2.

Superficial dissection of ventral part of the neck. The sternum has been cut through along its length and the lungs are seen between the two halves. The two sterno-cephalici and sterno-thyro-hyoidei have been separated in the middle line to show the pericardium. A band of fascia is seen running forwards from the apex which almost completely hides the trachea.

ference at the auriculo-ventricular groove was 9½ inches. The left side was occupied very largely by the left ventricle. This was 6 inches long and it extended about an inch beyond the anterior end of the right ventricle to form the apex of the heart. Above the posterior part of this ventricle on this side appeared part of the right ventricle with the pulmonary artery running horizontally backwards from it. Below the pulmonary artery lay the left auricular appendix, whilst behind this latter was the coronary sinus. This was excep-

tionally large, about 1 inch in diameter, and it ran downwards and forwards to the left of the posterior vena cava (to which it was adherent) to open on the floor of the right atrium. Immediately behind its entrance into the right auricle, the coronary sinus received the right coronary vein, the left vein opening an inch behind this. The right side of the heart showed in its anterior two-thirds the right ventricle, with the apex of the left ventricle projecting a short distance beyond this. The right inter-ventricular groove lay about the middle of the ventral surface, the left groove being situated towards the left of the dorsal surface. The grooves met, however, on this right surface about 1 inch behind the apex of the organ, forming a distinct *incisura apicis*. The posterior third of this surface was occupied by the right auricle, the atrium below, and the appendix extending above this on to the upper surface. Into the atrium, posteriorly, opened the posterior vena cava. This opening was situated in the middle line. Between the posterior vena cava and the right auricular appendix opened the right anterior vena cava.

The interior of the heart showed no marked peculiarities except in the right auricle. Both auriculo-ventricular valves were normal, as also were the pulmonary and aortic valves. The right ventricle showed a number of trabeculæ carneæ and a large moderator band. In the left ventricle there were a number of these bands which were slender and branching. The cavity of the left auricle was relatively very small, particularly its atrium, which lay compressed between the termination of the posterior vena cava and the upper part of the coronary sinus. The right auricle was of a peculiar shape. The auricular appendix was situated antero-superiorly (the heart being viewed in the position in which it lay in the animal), the atrium lying below and behind this. On the roof of the atrium was the large, somewhat oval opening of the right anterior vena cava. Below this, and on the inter-auricular septum, was the patent foramen ovale, partly closed, however, by a fenestrated membrane. At the back of the cavity, and separated from the right anterior vena caval opening above and in front by a thickened muscular ridge, was the opening of the posterior vena cava. Anteriorly there was a large auriculo-ventricular opening, whilst behind this, on the floor of the cavity, was the entrance of the coronary sinus.

There were five pulmonary veins, one, much larger than the others, being situated in the middle line.

The pulmonary artery ran horizontally backwards from the right ventricle. It supplied small branches, one for each lung, but the ductus arteriosus, a large vessel, appeared to be a direct continuation of the artery to the common aorta, so that these three vessels ran in a straight line backwards at the roof of the thorax towards the diaphragm. The ductus arteriosus was crossed on its upper and left aspects by the left axillary artery, whilst behind this lay the left anterior vena cava. There was no anterior aorta, as is usually found in bovines. The first branch of the common aorta after the coronary arteries was the innominate artery, the left axillary artery being detached a little

behind this at the level of the ductus arteriosus. The innominate (brachiocephalic) artery was long and somewhat bulbous in its early part. It took a forward course beneath the longus colli muscles and curved to the right across the middle line on a level with the right auriculo-ventricular groove. It detached in the middle line the left and right common carotid arteries, almost at the same point, and then continued as the right axillary artery. The thymus gland lay below the vessel. The common carotid arteries diverged early as they ascended the neck, the right soon gaining the outer face of the trachea with the vago-sympathetic trunk and the internal jugular vein above it, and the left occupying a similar position, but separated from the trachea by the heart and thymus gland. The right axillary artery ran backwards and to the right, crossing below the trachea and above the vago-sympathetic trunk and the internal jugular vein. The vessel was covered outwardly by the continuation of the aponeurosis of the sterno-cephalicus, and, curving downwards, at the entrance to the chest it pierced this aponeurosis and entered the axilla. In its course towards the axilla the artery gave off a common trunk for the dorsal and superior cervical arteries, then the vertebral artery, and lastly the internal thoracic artery. The left axillary artery, detached about 1 inch behind the innominate artery, took a course outwards above the ductus arteriosus, detaching a common trunk for the dorsal, superior cervical and vertebral arteries, then a

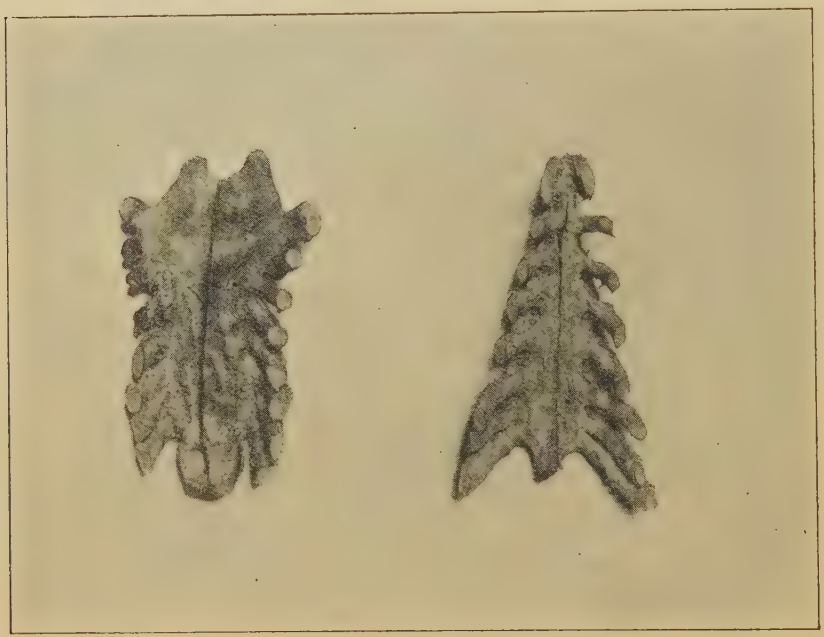

Fig 3.

Sternum of Calf the subject of ectopia (left) *and normal sternum.* Both specimens have been incised longitudinally and the xiphoid cartilage of the normal specimen has been removed.

little later gave off the inferior cervical and internal thoracic arteries, when it passed in front of the first rib into the axilla as on the right side.

The posterior vena cava passed upwards and backwards from the most posterior part of the base of the heart to reach the diaphragm. The vessel was enclosed in its special fold of pleura, but its association with the right phrenic nerve was short, for this nerve, gaining the thorax by passing between the right axillary artery and the right anterior vena cava, ran over the posterior part of the pericardium and the termination of the posterior vena cava, then backwards to the right of, and below, that vessel, enclosed in a fold of pleura derived from the outer side of the caval fold, to enter the diaphragm below the foramen dextrum.

The anterior venæ cavæ were both short vessels, but of large diameter. On the left side the external jugular and axillary veins united outside the aponeurosis in front of the first rib, and, running through the aponeurosis below the axillary artery, the vena cava reached the base of the heart, where it entered the coronary sinus. At this point the vena cava was joined by the internal jugular vein and a common trunk for the vertebral and superior cervical veins and the dorsal vein. Immediately behind the last-named the hemiazygos vein joined the coronary sinus. The right anterior vena cava, formed as on the left side by the union of the external jugular and axillary veins, soon passed through the fascial sheet in front of the first rib and became much increased in diameter as it was joined by the internal jugular vein and the common trunk of the vertebral, superior cervical and dorsal veins. It continued for a distance of about $1\frac{1}{2}$ inches inwards behind the right auricular appendix and opened into the atrium.

The thoracic duct passed from the dorsal to the ventral face of the aorta at the level of the fourth dorsal vertebra, then continued forwards beneath the aorta over the root of the left lung and across the upper aspect of the left pulmonary artery, to enter the left anterior vena cava near its opening into the coronary sinus.

The trachea occupied a normal position in the first few inches of its course, but on approaching the level of the apex of the heart it became pushed over to the right side and during the remainder of its course in the neck it lay above, and to the right of, the heart. Immediately behind the heart, *i.e.*, in the anterior part of the thorax, it inclined towards the middle line and divided.

The œsophagus was situated on the left of the trachea in the neck, but became pushed towards the right on approaching the heart. In the thorax it resumed its normal position to the right of, and later below, the aorta.

The thyroid bodies were not equally developed. The right was considerably the larger, having a length of 15 mm. The left was only 8 mm. in length. No deviation from the normal structure was observed in either body in sections under the microscope. The parathyroid glands also appeared normal.

As might be expected, the thoracic and cervical fasciæ were peculiarly disposed. These layers of fascia are always thick and strong in bovines, but owing to their intermingling in the neck in this instance the resulting sheets were exceptionally thick. It is to be noted that there was no connective tissue adhesion between the pericardium and the ventral layer of muscles or the skin, such as was described

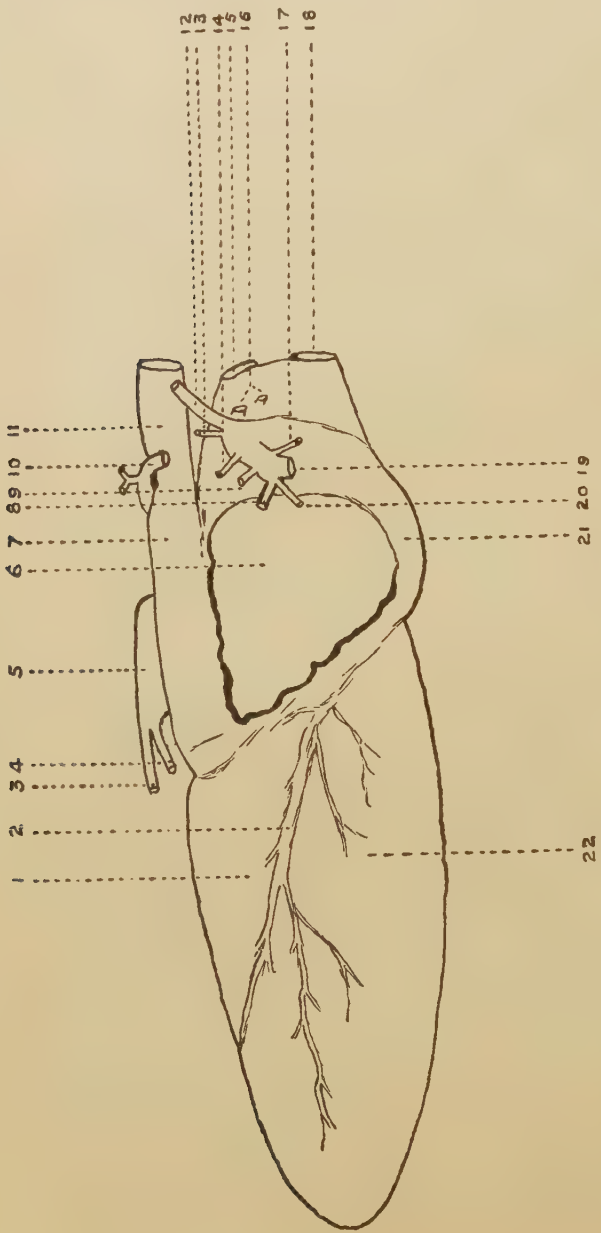

FIG. 4.

Ectopic Heart (left view). 1, Right ventricle; 2, Left interventricular groove; 3 and 4, Left and right common carotid arteries; 5, Innominate artery; 6, Left auricle; 7, Pulmonary artery; 8, External jugular vein; 9, Internal jugular vein; 10, Left axillary artery; 11, Aorta; 12, Hemiazygos vein; 13, Dorsal vein; 14, Common trunk of superior cervical and vertebral veins; 15, Pulmonary vein; 16, Branches of pulmonary artery; 17, Internal thoracic vein; 18, Posterior vena cava; 19, Left axillary vein; 20, Inferior cervical vein; 21, Coronary sinus; 22, Left ventricle.

as occurring in Handley's case. The pericardium, however, was attached along its length to the vertebral layer of cervical fascia, and from its apex bands ran forwards to blend with the fascia covering the parotid and submaxillary glands.

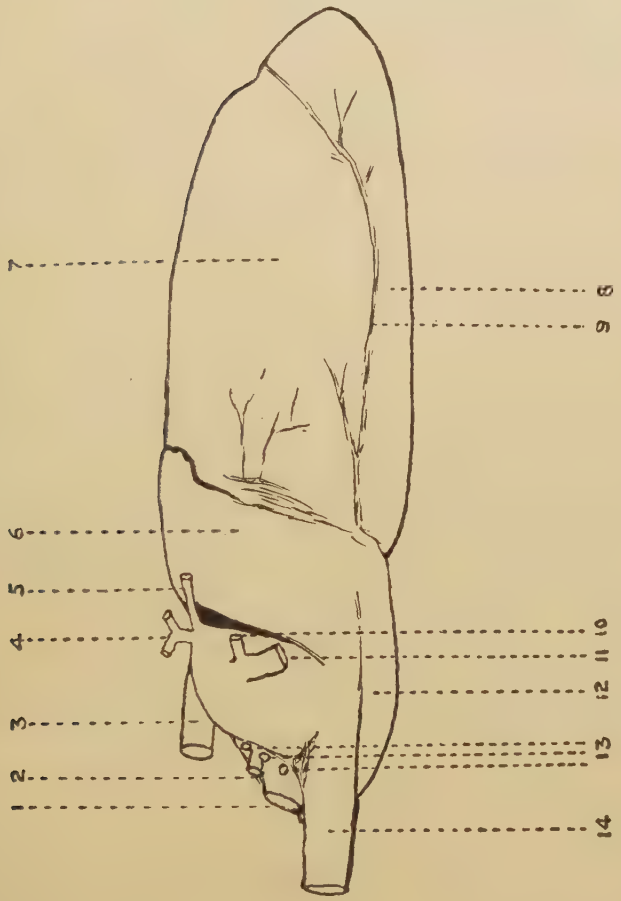

FIG. 5.

Ectopic Heart (right and inferior view). 1, Pulmonary vein; 2, Branch of pulmonary artery; 3, Aorta; 4, Common trunk of dorsal, superior cervical and vertebral veins; 5, Internal jugular vein; 6, Right auricle; 7, Right ventricle; 8, Left ventricle; 9, Right interventricular groove; 10, External jugular vein; 11, Right axillary vein; 12, Coronary sinus; 13, Pulmonary veins; 14, Posterior vena cava.

Conclusions.

The etiology of ectopia cordis is still undetermined. It is generally regarded as being a retention of the heart in its embryonic situation close behind the head. From the description already given of this case, however, it will be evident that the base of the heart at least lies within the chest. In fact, it would appear that if the apex of the heart were turned backwards instead of forwards towards the head, the position of the organ would be almost normal. One is led to believe then that the heart has rotated around a transverse axis with its apex turning forwards instead of backwards.

The abnormal structure of the sternum is noteworthy. Cases of ectopia of the heart have been reported as occurring simultaneously with the condition of cleft sternum (Class B3 of Sidney Jones). The widely separated centres of ossification in the anterior part of this structure and the indistinct nature of the pretracheal muscles near their origin from the sternum suggest that the closing in of the lateral walls of the embryo has been delayed, and is indeed incomplete, and that a definite fissure has only just been averted. If this is the case, then the depression on the upper face of the sternum would accommodate the apex of the heart whilst the organ was situated with its long axis lying in a vertical direction. This view is confirmed by the presence of the sterno-pericardiac ligaments which are attached to the sternum just behind the depression.

The rotation of the heart would be encouraged by two factors :—

1. Traction on the posterior part of the base. This would be associated with the backward movement of the diaphragm.

2. Systole of the heart. During systole the curve of the aorta tends to straighten out, and the apex of the heart moves forwards. In this case little resistance would have been offered to the straightening-out process and, this continuing, the long axis of the heart and the aorta would come to lie in practically the same straight line.

The double anterior vena cava occurs in the cases reported by both Van de Pas and Waterston—the only two cases in connection with which the writer has been able to trace any detailed description of the associated vessels.

Waterston, discussing the causation of the anomaly, suggests that a deficiency of the right venous valve of the sinu-atrial opening would encourage persistence of the left horn of the sinus and of the left duct of Cuvier. It must be borne in mind, however, that in bovines the coronary sinus is normally large, for it has opening into its left extremity the so-called hemiazygos vein which drains the blood carried by the azygos and hemiazygos veins of man, and which represents a persistence of the upper part of the left posterior cardinal vein. Harris and Whitney[14] go further, and state that if the valve is absent from the coronary sinus the latter receives systemic blood from the anterior cardinal or azygos systems. But in these cases of double anterior vena cava the abnormality consists not in the absence of a Thebesian valve with its associated persistence of the

embryonic left duct of Cuvier, but in the further persistence of the posterior (or lower) part of the anterior cardinal vein opening into the duct. The complete separation of the right and left sides of the venous system is what would be expected in view of the suggestion that there has been in this case a failure of the lateral walls of the embryo to meet completely.

It should be mentioned that abnormalities of the thyroid gland are known to be associated with abnormalities elsewhere, and it is to be noted that the left gland in this animal was considerably smaller than the right. The writer failed, however, to observe any factor, such as excessive pressure on the body, which might account for this peculiarity.

Acknowledgments.

The writer desires to express his gratitude to Professor Share Jones, in whose department the dissection was carried out, for his valuable guidance and advice, and also to Dr. T. J. Jones for much helpful criticism.

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CHARACTERS OF HÆMOLYTIC STREPTOCOCCI ISOLATED FROM PATHOLOGICAL CONDITIONS IN FOWLS*

By Philip R. Edwards,

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There are a number of references in the literature to the isolation of streptococci from various diseases of chickens. As a rule these streptococci are indefinitely characterised, so that the reader is unable to determine to what group the organisms in question belong. Several cultures from avian sources have been examined by the writer, and the present paper is a report of this work.

In previous papers (Edwards 1932, 1933, 1933a) on the low-acid-producing, non-sodium-hippurate-hydrolysing streptococci of animal origin, it was demonstrated that these organisms differed in their biochemical properties from streptococci of human origin. The results can best be expressed in tabular form and are given in Table I. It may be seen that *Str. equi* differed from all other cultures

TABLE I.

Biochemical Characters of Human and Animal Streptococci.

Source.	*Number of Cultures.*	*Lactose.*	*Sorbitol.*	*Trehalose.*	*Reduction of Methylene Blue.*
Animal—					
Str. equi	16	—	—	—	—
Type A	159	+	+	—	—
Type B	7	±	—	+	+
Human	120	±	—	+	1— 6+

studied in that it fermented neither lactose, sorbitol, nor trehalose. This type was found only in strangles of horses. Of the remaining animal streptococci, 96 per cent. (type A) fermented sorbitol and failed to attack trehalose, while cultures of human origin uniformly produced acid from trehalose but did not ferment sorbitol.

The small group of animal streptococci (type B) which resembled the human cultures in their action on sorbitol and trehalose regularly

*The investigation reported in this paper is in connection with a project of the Kentucky Experiment Station and is published by the permission of the Director.

reduced methylene blue, while only 5 per cent. of the human strains were able to decolourise the dye.

In addition, Lancefield (1933) demonstrated that *Str. equi* and the sorbitol-fermenting streptococci of animal origin could be distinguished from human streptococci by group precipitin tests, using acid extracts of the organisms as antigens. This fact was confirmed by Edwards (1934). It was found that *Str. equi* and the type A and type B animal streptococci possessed a common antigen which was lacking in streptococci of human origin. Likewise human streptococci possessed an antigen not found in animal streptococci. These differences in biochemical and serological behaviour furnished an accurate method for the differentiation of hæmolytic streptococci of human and animal origin.

Materials and Methods.

The methods used in studying the biochemical characters of the organisms were those employed by Edwards (1933). The method of Lancefield (1933) was used in the precipitin tests. The cultures to be reported on are as follows :—

3,962, 6,554, 17,090, 17,098, 18,784, 21,013, 22,499, 23,155—Isolated from tibia of chickens affected with " slipped tendon."*

Six of these cultures were received from Dr. R. Graham. The remaining two were isolated by the writer.

H—Isolated by Dr. C. B. Hudson from apoplectiform septicæmia of chickens.

GI, GII, GIII—Isolated by Dr. C. S. Gibbs from bronchitis of baby chicks.

Results.

All the cultures were found to belong to the group of low-acid-producing streptococci. None of them produced an acidity exceeding pH 4·8 in glucose broth. None of the organisms hydrolysed sodium hippurate and all produced large amounts of hæmolysin in a fluid medium. Further biochemical characters are given in Table II. The results of the serological tests are set forth in Table III.

Discussion.

A. Cultures from Slipped Tendon.

Certain chickens affected with slipped tendon have, in addition to the deformity of the bones of the leg, a chronic arthritis or periostitis. It was from such chickens that the cultures under consideration were isolated. These streptococci probably are of little or no

*" Slipped tendon " is a term used to describe the condition resulting from a slipping of the tendon of Achilles from its position, due to a deformity of the tibia. Chickens so affected rest upon the tarsus. The disease is thought to be the result of improper nutrition.

TABLE II.

BIOCHEMICAL CHARACTERS OF STREPTOCOCCI ISOLATED FROM CHICKENS.

Culture.	*Lactose.*	*Sorbitol.*	*Trehalose.*	*Reduction of Methylene Blue.*
3,962	+	+	—	—
6,554	+	+	—	—
17,090	+	+	—	—
17,098	+	+	—	—
18,784	+	+	—	—
21,013	+	+	—	—
22,499	+	+	—	—
21,355	+	+	—	—
H	+	+	—	—
GI	+	—	+	—
GII	+	—	+	—
GIII	+	—	+	—

TABLE III.

SEROLOGICAL CHARACTERS OF STREPTOCOCCI ISOLATED FROM CHICKENS.

	Precipitation with Antisera for			
Antigens.	*Str. equi.*	*Animal Type A.*	*Animal Type B.*	*Human.*
3,962	+	+	+	—
6,554	+	+	+	—
17,090	+	+	+	—
17,098	+	+	+	—
18,784	+	+	+	—
21,013	+	+	+	—
22,499	+	+	+	—
23,155	+	+	+	—
H	+	+	+	—
GI	—	—	—	+
GII	—	—	—	+
GIII	—	—	—	+

importance in the ætiology of the disease. They probably gain entrance only after primary damage to the tissues has occurred. Nevertheless their presence is of interest and must be taken into consideration when the pathology of certain cases is studied. They apparently cause degenerative changes not found in uncomplicated cases of the disease.

By comparing the results obtained with these cultures with Table I it is evident that they are typical animal streptococci. Both their biochemical and serological properties are characteristic of the type of streptococcus frequently associated with diseases of the lower animals.

B. Streptococci from Apoplectiform Septicæmia.

Several workers have reported a highly fatal, septicæmic disease of chickens caused by a streptococcus. The organism responsible has not been well characterised in any of the papers and there are conflicting reports regarding certain properties.

Norgaard and Mohler (1902), Magnusson (1910), and Hudson (1933) reported that the streptococcus responsible for this disease was non-capsulated. Dammann and Manegold (1905), Bergman (quoted by Magnusson), and Greve (1908) have observed distinct capsules on streptococci isolated from septicæmia of fowls. Bergman noted that the streptococcus was Gram-negative, while the other workers reported it as Gram-positive. Magnusson found that the organism fermented dulcitol, sorbitol, and mannitol, while, according to Hudson, none of these substances were attacked. Such contradictions indicate the need for an exact study of the streptococci responsible for the disease.

Unfortunately, only one culture was avilable for study, the strain isolated and described by Hudson (1933). While Hudson reported that the organism did not ferment sorbitol, it has been found upon repeated trials to ferment this substance quite readily. Different samples of sorbitol yielded identical results, and acid was produced within 24 hours in each instance. Trehalose, dulcitol, and mannitol were not attacked. These results, together with the serological characters given in Table III, identify the organism as a type A animal streptococcus.

It has been demonstrated (Edwards, 1933) that members of this group of streptococci regularly produce capsules. As would be expected, the organism in question possesses large, distinct capsules. These can be demonstrated quite readily in moist India ink preparations of young cultures on blood agar. Until further evidence is available, the streptococcus of apoplectiform septicæmia of chickens may be regarded as a member of the group which has been designated type A. Further work is required to determine whether or not organisms of this group isolated from different species of animals are distinct. No method has been devised by which the group can be subdivided.

C. Streptococci from Infectious Bronchitis.

Gibbs (1933) described hæmolytic streptococci isolated from baby chicks affected with bronchitis. These organisms, designated *Str. bronchitis*, were compared with culture designated as *Str. pyogenes* and *Str. epidemicus*. According to Gibbs' report, the streptococci isolated from chicks differed from *Str. pyogenes* and *Str. epidemicus* in the following respects : *Str. bronchitis* was Gram-negative and the other cultures were Gram-positive. *Str. bronchitis* fermented raffinose while *Str. pyogenes* and *Str. epidemicus* did not.

Str. bronchitis and *Str. epidemicus* did not attack levulose, while *Str. pyogenes* fermented this sugar. *Str. bronchitis* fermented inulin, *Str. pyogenes* and *Str. epidemicus* did not ferment this substance. Furthermore, *Str. bronchitis* differed from *Str. pyogenes* and *Str. epidemicus* in agglutination and agglutinin absorption. Finally, Gibbs (1933) found that recently isolated strains of *Str. bronchitis* were pathogenic for baby chicks but that *Str. pyogenes* and *Str. epidemicus* were not.

Three cultures isolated from baby chicks were received from Dr. Gibbs. His findings have not been confirmed. The cultures are Gram-positive streptococci which ferment levulose and fail to ferment raffinose and inulin. It may be remarked in passing that the fermentation of inulin is a property foreign to the hæmolytic streptococci, and the fermentation of this substance has been generally used to differentiate streptococci and pneumococci.

While Gibbs (1933) states that streptococci derived from bronchitis in chicks differ in agglutination and agglutinin absorption from *Str. pyogenes* and *Str. epidemicus*, he fails to take into consideration the fact that there is no established agglutinative type representative of these species. Hæmolytic streptococci grouped together as *Str. pyogenes* represent a number of heterologous agglutinative types. Insufficient work has been done on the culture classified as *Str. epidemicus* to establish their antigenic relations.

The cultures received from Dr. Gibbs ferment trehalose, fail to attack sorbitol, and do not reduce methylene blue. They possess the biochemical characters of human streptococci. Furthermore they precipitate with antiserum prepared from streptococci of human origin and fail to react with antisera prepared from animal strains.

The cultures GI, GII and GIII, possess all the attributes of human streptococci. Nothing can be said of them except that, in the light of our present knowledge, they cannot be distinguished from streptococci of human origin.

Summary.

Hæmolytic streptococci isolated from cases of slipped tendon and from apoplectiform septicæmia of chickens were shown to be typical animal streptococci. Hæmolytic streptococci said to have been isolated from bronchitis of baby chicks possessed the characters of streptococci of human origin.

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SWINE FEVER AND PARATYPHOID IN PIGS.

By Max Henry,

Chief Veterinary Surgeon, New South Wales.

In an editorial headed : " Swine Fever and Paratyphoid in Pigs " (*Jr. Comp. Path. & Ther.*, vol. xlvi, Part 4, pp. 257-265), reference is made to an article by Hindmarsh and Edgar published in Veterinary Research Report, No. 6, Department of Agriculture, N.S.W. In commenting on this article, the following statement appears :—

> " Without losing sight of any of the evidence in the article, we cannot agree that the possibility that the original disease in the abattoir pigs was swine fever was excluded."

It is desired herein to produce evidence from the administrative and epizootiological side supporting Hindmarsh and Edgar in their conclusions.

It is necessary in the first place to consider the history of swine fever in New South Wales. The first recorded evidence of the existence of swine fever is in 1902, when there suddenly occurred a very serious epizootic in the piggeries around the seaport of Sydney. This outbreak was eventually stamped out, although for some years cases of what were in those days regarded as swine fever continued to appear, but the correctness of the diagnosis is very doubtful. No biological tests were, however, carried out until 1924.

From 1923 onwards, no case of swine fever was detected in New South Wales until the sudden appearance of the disease in 1927. The details of that outbreak are fully set out in the report by Henry, Seddon and Blumer (Department of Agriculture, N.S.W.—The Swine Fever Outbreak of 1927-28 in N.S.W.).

The reasons—which are held to be conclusive—why it is considered New South Wales had prior to that outbreak been free from this disease for a number of years are set out, as well as the reasons leading to the conviction that the disease had been freshly introduced from Victoria. At the same time, outbreaks of swine fever occurred in Tasmania and South Australia—in each case traceable to Victorian pigs.

For some years prior to this outbreak all Australian States had regularly reported freedom from swine fever, with the exception of one isolated alleged outbreak in Kalgoorlie, Western Australia. Now the geographical situation of Kalgoorlie makes this diagnosis very dubious. Thus we find in a reputedly disease-free Continent four States suddenly attacked, and all tracing to a central infection in Melbourne, Victoria.

The outbreaks of 1927-28 was completely cleaned up in those years—the last recorded outbreaks being Victoria, August, 1928 ; Tasmania, June, 1927 ; South Australia, November, 1928, and New South Wales, November, 1928. Since that date all States

in Australia report consistently freedom from swine fever and there appears no reason to doubt the correctness of that report.

So far as New South Wales is concerned, the control exercised is considered to be such that the occurrence of swine fever would not be overlooked for long. The environs of Sydney obviously constitute the danger zone in this State. In those areas every piggery is regularly inspected by Veterinary Officers and Inspectors of Stock. The outlet for the Sydney pigs is the Homebush Sale Yards. Every sale there is attended by a Departmental Officer. The vast majority of these pigs are slaughtered at Homebush Abattoirs. The Veterinary Officers of the Meat Board on duty there have not reported any case of Swine Fever since 1928.

Inspection of piggeries to a less intense degree is carried on throughout the State.

In both New South Wales and Victoria compensation is paid for pigs slaughtered on account of the presence of swine fever or condemned at the Abattoirs for that cause. In New South Wales certainly, and it is understood in Victoria also, no claim for pigs slaughtered since 1928 has been made on account of the presence of swine fever.

It is held that the pigs regarding which Hindmarsh and Edgar reported were pigs produced in a swine fever-free continent: not from a swine fever-free farm or even area. These pigs did not come from farms on which mortalities resembling swine fever had occurred. In fact, there is no evidence that any particular mortality was occurring. On the other hand, they mostly were pigs which had been subjected to a long railway journey. Similar cases are still occurring, and although many of the holdings despatching these pigs have been specially inspected, no sign of swine fever can be detected. Quite a number have come from a particular piggery within the abattoir area which is regularly inspected and which is remarkably free from mortality. In this piggery breeding is not carried out—the pigs mostly are purchased at distances ranging up to 580 miles distant and are brought down by rail and then topped off for a longer or shorter period according to the conditions in which they arrive.

It is considered that we have here an instance in which the evidence of the administrative side is of outstanding importance.

Note by the Editor.

The efficiency of the administration of the regulations dealing with swine fever in Australia does not really arise in connection with the editorial article in this Journal to which Mr. Henry refers, and it was not the intention of its author to affirm that swine fever exists in any part of that continent at the present time. It must be pointed out, however, that Hindmarsh and Edgar did not share the Chief Veterinary Surgeon's complete confidence that the disease among the pigs at the Homebush abattoir could not be swine fever. The actual words used by them were: " In view of the fact that swine fever had been present in this State some years previously, it was necessary to eliminate the possibility of the lesions produced in experimental animals being due to this disease, and to show that the bacillus isolated was

capable of causing lesions similar to those in pigs condemned at the abattoirs."

These words were interpreted to mean that Messrs. Hindmarsh and Edgar considered it necessary to prove that the disease was not swine fever, and the criticism of their investigations was directed to the fact that they failed to take the action that wou.d have given an unequivocal answer to the question. Whether in any suspected case a pig is or is not infected with swine fever cannot be proved by cultivating or failing to cultivate organisms of the *B. suipestifer* type from the animal's blood or organs. It was, therefore, not proved in the case in question that the disease was not swine fever. In a case in which the lesions raise a suspicion of swine fever the proper course is not to determine whether organisms of the *B. suipestifer* type can be cultivated from the body, but to employ the biological test to show whether a filtrate from the blood or organs will infect pigs with that disease.

REVIEWS.

Veterinary Hygiene. By R. G. Linton, PH.D., M.R.C.V.S. Second Edition. Edinburgh, W. Green and Son, 1934. Price 21s. net.

The new edition of Professor Linton's excellent textbook shows only minor alterations, the chief of which are that the section on the preventable diseases deals only with those that are notifiable in Great Britain and chapters have been added on the housing of poultry and the most important contagious diseases of fowl.

The work is well designed to meet the requirements of veterinary students and must do much to lessen the labour of teachers.

An Introduction to Practical Bacteriology. A Guide to Bacteriological Laboratory Work. Fourth Edition. By T. J. Mackie, M.D., D.P.H., and J. E. McCartney, M.D., D.SC. Edinburgh, E. and S. Livingstone, 1934. Price 12s. 6d. net.

The fact that this small work has reached a fourth edition in nine years is evidence that it is well adapted to the requirements of medical students. The text is about equally divided between bacteriological technique and concise description of the pathogenic micro-organisms, including the viruses. In fact, however, the work conveys much more information regarding the bacterial diseases than is suggested by its title, but nothing that can be considered redundant. The paper, type and binding are good.

The Toxicology of Plants in South Africa together with a Consideration of Poisonous Foodstufs and Fungi. By Domo G. Steyn, B.SC., DR.MED. VET. (VIENNA), D.V.SC. South Africa Central News Agency, Ltd., 1934. Price £2 7s. 6d.

This work may be said to be the fruit of the author's prolonged studies of a rich material in connection with the poisonous plants of South Africa. It is a valuable contribution to the literature on the toxicology of plants in general, and will prove an indispensable work of reference to all those whose interests are involved in plant poisoning in South Africa. With the index, it extends to 609 8vo pages and contains 135 useful illustrations.

The first 200 pages deal in a general way with such subjects as absorption and modes of action of poisons ; diagnosis, prognosis and treatment of plant-poisoning ; and eradication of poisonous plants. The remainder of the work treats separately of each of the poisonous plants found in South Africa, many of which, of course, also occur in other countries. The publisher's share of the work has been very well done.

CONTENTS

GENERAL ARTICLES.

PAGE

"Immune" and "Hyperimmune" Cattle Plague Antiserum. By S. C. J. Bennett 163

The Incidence in Great Britain of *Br. abortus* Infection in Various Domestic Animals as Indicated by the Agglutination Test. By F. W. Priestley 181

The Role of *Salmonella suipestifer* in the Production of an Enteric Disease Independent of Hog Cholera Virus. By H. E. Biester 190

Canine Distemper Vaccine. Two Experiments with Vaccine prepared by Photodynamic Inactivation of the Virus with Methylene Blue. By Thomas F. Dempsey and Victor Mayer 197

Further Observations on Cell Inclusion Disease of Fowls, and Differential Diagnosis from Fowl Plague. By S. J. Gilbert and G. B. Simmins 201

Examination of the Brachial Plexus of Normal Fowls for Lymphoid Infiltrations. By V. Elvira Palmer and Jacob Biely ... 205

Preliminary Note on the Transmission of Bovine Hæmorrhagic Septicæmia by the Flea *Ctenocephalus felis* Bouche. By R. Daubney and J. R. Hudson and J. I. Roberts... 211

A Case of Spontaneous Tuberculosis in a Goat. By Herta Schwabacher 214

A Note on Two New Blood Parasites of Cattle, Eperythrozoon and Bartonella. By S. Adler and V. Ellenbogen 219

ABSTRACTS.

No-Lesion Cases in Tuberculin Testing of Cattle in the United States. By E. G. Hastings, Walter Wisnicky, B. A. Beach and Jennet McCarter 222

Equine Encephalomyelitis. By Edward Records and Lyman R. Vawter 229

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